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Trauma Induced Coagulopathy

Hunter B. Moore
Ernest E. Moore
Matthew D. Neal
Editors

Second Edition

Springer

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HBM dedicates this book to his wife Brooke A Moore and Baby Estelle.

MDN: with love and gratitude to my "first family" Ellie, Cameron, and Donielle, and to my "second family" at UPMC Trauma built by the consummate surgeon, scholar, and family man, Andrew B. Peitzman, MD.

EEM dedicates this book to his wife Sarah Van Duzer-Moore, MD; son Peter K. Moore, MD, and his wife Tiffany Tello, MD; and of course other son Hunter B. Moore, MD, PhD, and his wife Brooke A Moore, MA.

HBM and EEM also dedicate this second edition to Eduardo Gonzalez for his editorial contribution to the first edition of this book. We wish him the best of luck in his academic plastic surgery career. We will be forever grateful for his monumental efforts in helping put the first edition together and never forget the countless hours spent in Steamboat cabin editing what seemed to be an endless list of chapters.

Preface – 2nd Edition

Like many good ideas in clinical medicine, *Trauma Induced Coagulopathy* was the product of a multidisciplinary research meeting. As the process unfolded, multiple classic papers were identified that addressed different concepts of coagulation changes following injury. It soon became apparent that consolidation of all of these concepts was too large for practical distribution or synthesis into a review article. We agreed the most useful reference would be a text of chapters written by those conducting research in various fields related to coagulation resulting in the first edition of *Trauma Induced Coagulopathy* in 2016. With the growing interest in understanding and managing coagulation in trauma, an updated second edition was due. During this interval, the Trans-Agency Consortium on Trauma Induced Coagulopathy (TACTIC), supported by the National Institutes of Health and coordinated with clinical trials funded by the Department of Defense, made significant efforts to enhance multidisciplinary research in trauma resulting in countless new discoveries. International collaboration and consensus resulted in a definition of TIC proposed by a working group of the International Society of Thrombosis and Hemostasis (ISTH). Several large clinical trials were also completed during this time, in addition to new clinical devices for measuring coagulation. The second edition has been expanded to 46 chapters from its original 35 to incorporate the massive global efforts in understanding, diagnosing, and treating trauma induced coagulopathy. At the time of publication, the world is focused on the vexing problem of COVID-19, and we have rapidly come to realize that a profound hypercoagulable state markedly contributes to morbidity and mortality. Knowledge gained from our collaborations and summarized in this text is now being deployed against the COVID-19 associated coagulopathy. At the same time, uncontrolled hemorrhage remains the leading cause of preventable death following trauma throughout the world, and the driving mechanisms remain to be established.

Aurora, CO, USA — Hunter B. Moore
Pittsburgh, PA, USA — Mathew D. Neal
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Part I

Introduction

1 Historical Perspective of Trauma-Induced Coagulopathy

Ernest E. Moore and Hunter B. Moore

Injury is the leading cause of death worldwide [1], and the third leading cause of mortality in the United States [2]. Despite advances in emergency medical systems and trauma care, deaths from injury have increased in the United States over the last decade [3]. In both the civilian [4] and military [5] settings, uncontrolled hemorrhage is the leading cause of preventable death after injury. In civilian studies, 80% of deaths from hemorrhage occur within the first 6 h, at a median time of 2.5 h [4]. Consequently, there is intense interest worldwide in the pathogenesis of coagulopathic bleeding after injury and its early management. While there have been substantial insights, the words of Mario Stefanini in his address to the New York Academy of Medicine in 1954 [6] remain applicable today: "The ponderous literature on the subject of hemostasis could perhaps be considered a classical example of the infinite ability of the human mind for abstract speculation. For several years, the number of working theories of the hemostatic mechanisms greatly exceeded and not always respected the confirmed experimental facts. In recent years, however, the revived interest in this field has led to an accumulation of new findings, which has been almost too rapid for their orderly incorporation into a logical working pattern. As a result, we have rapidly gone from a state of orderly ignorance to one of confused enlightenment, from which we have not emerged as yet."

The evolution of our understanding of the complexities of coagulopathy associated with trauma has been, in large part, the result of collaboration between civilian and military investigators and clinicians. Observations during war, due to a concentrated experience, often stimulate research in civilian academic centers, which culminates in advances in our patient care and understanding the fundamental problem. The earliest reports of coagulopathy in injured patients were generated from military research teams, often including civilian consultants, during major wars. These novel observations would then intensify hemostasis research in civilian centers. Ultimately, the resulting findings improved coagulopathy management in subsequent conflicts, and primed the environment for making new observations. The specific contributions to our understanding of coagulopathy, however, are somewhat difficult to ascertain from World War I through Vietnam because the primary focus was on optimizing shock resuscitation at a time when plasma or whole blood was employed to replace acute blood loss [7]. Nonetheless, several landmark contributions are well recognized.

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In 1916 the US National Research Council formed a Subcommittee on Traumatic Shock that collaborated with the British Medical Research Committee to study wounded soldiers in the front lines of France. Among them was Walton B. Cannon from Harvard, who was perplexed by the inconsistencies of the prevailing toxin theory of shock. Based on observations made on the battlefield in France during 1918 [8], Cannon wrote "Whatever the nature of the bodily changes…the heart, nervous system and other organs are suffering from an insufficient blood supply" and later admonished "if the pressure is raised before the surgeon is ready…blood that is sorely needed may be lost." Cannon documented the dynamic nature of coagulation experimentally with epinephrine infusion into animals that provoked hypercoagulability followed by hypocoagulability [9]. Cannon also stated prophetically "…shock is a loss of homeostasis, and without homeostasis the patient does not survive." Interestingly, two pivotal discoveries made during the later years of WWI [10], Richard Lewisohn's demonstration that sodium citrate was a safe process to store blood in 1915 and Karl Landsteiner's discovery of major blood types in 1917, led to the transfusion of blood to soldiers at the end of the war [11]. In 1936, based largely on Cannon's war observations and his own research at Vanderbilt and Johns Hopkins, Alfred Blalock [12] concluded "the work of recent years has shown that shock is dependent on an inadequate supply of blood to the tissues, which may be brought about by the most diverse causes," that is, hematogenic, neurogenic, vasogenic, and cardiogenic.

In the spring of 1940, with major victories achieved by Germany and Japan, the US involvement in the war appeared inevitable. Military experts recognized that bottled whole blood would be logistically impractical and enlisted the expertise of Edwin Cohen, a Harvard biochemist, to deconstruct blood in order to deliver its components to the battlefield [13]. Cohen was successful in purifying albumin as well as preparing plasma. At the onset of World War II, the National Research Council's Committee on Transfusion recommended that dried plasma—not blood—would be used if combat occurred outside the continental United States because it was easy to prepare and transport, whereas whole blood had to be typed, cross-matched, and refrigerated. However, based on the work of consultant Edward D. Churchill [14] in North Africa, who concluded, "wound shock is blood volume loss," the policy was changed to whole blood administration and implemented in Italy in 1943. In the preface to Colonel Churchill's review, Brigidar General Fred Rankin stated, "The present-day health standards of our troops and survival rate among our wounded have been unequalled in the history of war-fare. Perhaps one of the most important factors contributing to this highly record has been the role played by professional consultants."

In 1952, the Board for the Study of the Severely Wounded systematically reviewed the cause of death in 186 war casualties. The report was dominated by the discovery of a new syndrome "post-traumatic renal failure" that was attributed to prolonged hypoperfusion. This observation ultimately led to a paradigm shift in resuscitation, incorporating crystalloid as a fundamental component of initial fluid administration [15]. Contemporary studies in civilian hospitals, based on observations in trauma and burn patients, reported a "severe bleeding tendency" implicating fibrinolysis [16, 17]. The plasmin–antiplasmin system had been characterized at this point [18]. Alternatively, others postulated the loss of a labile clotting factor in whole blood and recognized the key role of platelets in hemostasis [19, 20]. In 1954, Stefanini [6] noted that post-injury hemorrhage persisting despite surgical control of bleeding was variously referred to as medical bleeding, diffuse bleeding diathesis, post-transfusion bleeding disorder, and disseminated intravascular coagulation (DIC), reflecting a general lack of consensus in the pathophysiology.

During the Korean War, William Stone is credited with promoting surgical research teams in the combat zone in Korea [21]. Scott and Crosby [22], representing one such team, reported that the prothrombin time (PT) was doubled in combat casualties while platelet count and fibrin-

ogen were increased. They also speculated that the cause was due to a labile clotting factor during blood storage. Artz and Fitts [23] observed that severely injured soldiers in the Korean Conflict required both return of shed blood and crystalloid for optimal survival, inspiring the later seminal work of Tom Shires [24] defining the scientific basis for crystalloids.

After the Korean War, civilian studies implicated a number of causative factors responsible for bleeding associated with major surgery requiring transfusion, including DIC [25], fibrinolysis [26], compromised viability of platelets in stored blood [27], and the loss of the labile factors V and VIII during storage [28]. The initial response to experimental hemorrhagic shock was hypercoagulability, followed by a progressive state of hypocoagulability with decreases in factors V, VIII, IX, X, and XI along with reduced fibrinogen and platelets [29]. The early clinical studies in Baltimore further identified a third phase of hypercoagulability in those who survived the intermediate period of hypocoagulability [30]. The authors concluded that in surviving patients, the oscillatory pattern converges into a "dynamic homeostatic state," whereas, in nonsurvivors, "fluctuations exceeded safe limits and behaved like a runaway system."

Based on the compelling experimental work by Shires et al. [24], the major change in resuscitation strategy in Vietnam was the administration of large volumes of crystalloid. This policy virtually eliminated acute kidney dysfunction, but led to a new entity coined "Da Nang Lung" [31], later termed the acute respiratory distress syndrome (ARDS) as the civilian counterpart [32]. The first large study on coagulation disorders in combat casualties from Vietnam was reported by Simmons et al. [33]. In their comprehensive analysis of 244 injured soldiers, the authors concluded that there is "an initial phase of hypercoagulability followed by hypocoagulability and this seemed best explained by DIC. Massive transfusion is accompanied by a dilutional coagulopathy compatible with factor levels in stored blood. Platelet levels fell, but PT, partial thromboplastin time (PTT), and fibrinogen levels are less affected. Fresh whole blood partially counteracts this dilutional state, but is rarely necessary." Miller et al. [34] studied 21 patients requiring a massive transfusion in Vietnam. Significant coagulation defects were not evident until 20 units of stored blood was administered. A dilutional defect in platelets appeared to be the primary cause for bleeding, and this was reversed with fresh whole blood administration. Interestingly, they reported no evidence of DIC or fibrinolysis. In 1974, John A. Collins [35] systematically reviewed the problems associated with massive transfusion and offered these observations: [1] "Early complete replacement of blood volume in the massively bleeding patient lessens the impact of exchange transfusion with stored blood," [2] "an intact circulation is a very good defense against the metabolic problems of massive transfusion," and [3] "historically as new problems associated with massive transfusion have been defined, they have almost always been grossly overstated."

Coagulation research in civilian institutions in the early 1970s began to elucidate the molecular events resulting in thrombin generation as the common end product of the extrinsic and intrinsic clotting pathways [36, 37]. In the clinical arena, trauma surgeons recognized that controlling bleeding from the liver was a priority to improve survival following trauma, but much of the work concentrated on techniques to achieve mechanical hemostasis with some mention of packing when bleeding continued [38–40]. It was also noted that tissue disruption from blunt trauma was associated with more problematic bleeding than penetrating wounds, stimulating resurgent interest in DIC and subsequent pulmonary microemboli [40, 41]. In the later 1970s, trauma surgeons began to recognize that bleeding following massive transfusion with stored blood required supplemental clotting factors. This literature is confounded by the fact that blood banks began to implement blood component therapy [42], a policy change that unmasked the prevalence of a trauma-related coagulopathy. In 1979, our group [43] and others [44–46] observed that the majority of patients succumbing to liver injuries died of a coagulopathy, after surgical control of bleeding. We recommended pre-emp-

tive fresh frozen plasma (FFP): "If the patients remain hypotensive after the second unit of whole blood, FFP should be administered then and with every fourth unit thereafter." Furthermore, we advocated fresh whole blood "…if bleeding persists despite normal PT, PTT, and bleeding times" [43]. Stimulated by these findings, we analyzed a group of patients who developed life-threatening coagulopathy with major vascular injuries and noted the compelling association of metabolic acidosis and hypothermia. Confirming the independent effects of acidosis and hypothermia on coagulation experimentally [47], we proposed the "bloody vicious cycle" in 1981 [48], which subsequently became known as the "lethal triad." The concept of truncating definitive repair of all injuries in coagulopathic patients in the operating room, to allow for correction of hypothermia, acidosis, and coagulopathy in the intensive care unit, was the fundamental basis for "damage control surgery" introduced by Harlan Stone et al. in 1983 [49]. In studying our coagulopathic injured patients in 1981 [48], we noted that higher ratios of FFP to stored blood were associated with improved survival and advocated presumptive FFP:blood administration of 1:4 in the emergency department. Charles Lucas and Anna Ledgerwood also conducted animal work that supported the concept of pre-emptive FFP during massive transfusion [50]. In the later 1980s [51], the Detroit General Group systematically studied coagulation abnormalities in patients requiring a massive transfusion of stored red blood cells (RBC) and postulated consumption of factors, reflected in standard measures of coagulopathy, that is, PT, PTT, and thrombin time (TT). Collectively, the coagulopathy associated with severe trauma was believed to be secondary to both consumption and dilution of clotting factors. There was also considerable interest in the early administration of platelets due to the long-term observation of deteriorating platelet numbers in stored blood, although clinical trials failed to confirm a benefit of pre-emptive platelet administration [52].

In the ensuing decade much of the clinical investigation centered on optimizing the use of damage control surgery for refractory coagulopathy [53–55]. Coagulation research during this period was further complicated by the practice of aggressive crystalloid resuscitation targeting supra-physiologic oxygen delivery, promulgated by William Shoemaker et al. [56]. This resulted in an epidemic of compartment syndromes, with much attention diverted to the urgent need to decompress the abdomen following protracted shock managed with high-volume crystalloid resuscitation [57]. In retrospect, most of the compartment syndromes and, to a large extent, coagulopathies were generated by overzealous infusion of crystalloid driven by the subsequently disproven concept of supra-physiologic oxygen delivery [58]. There is no question that chasing oxygen delivery with Swan-Ganz catheters and attempting to correct metabolic acidosis with large-volume crystalloid loading added a substantial component of dilutional coagulopathy [59].

The first decade of the twenty-first century perhaps represents the most significant insights gained into trauma-associated coagulopathy in modern history, and many of the contributing investigators are authors in this monograph. Progress was unquestionably inspired by the revolutionary concept of the cell-based model of coagulation proposed by Hoffman and Monroe [60] who emphasized the fundamental role of platelets as a platform for clotting factor assembly and thrombin generation on damaged endothelium. Paradoxically, these new insights led to the unbridled use of activated factor VII, which was ultimately proven unjustified [61, 62]. In 2003, MacLeod et al. [63] from the University of Miami made the observation that 28% of severely injured patients had an elevated PT on arrival to the hospital, and this was associated with an increased risk of mortality. At the same time, Karim Brohi [64] from the Royal London Hospital reported that 24% of severely injured patients had prolonged clotting times, and extended their analysis to demonstrate this abnormality was independent of fluid administration and, consequently, termed the syndrome the "acute coagulopathy of trauma" (ACOT). Stimulated by his observations on the ACOT in London, Brohi pursued a trauma research fellow-

ship with another fellow, Mitch Cohen, and colleagues in San Francisco. Together, in 2007, this civilian research team provided enticing evidence that activation of protein C is a mechanistic component of ACOT [65]. Shortly thereafter, Par Johansson [66] from Copenhagen added evidence of endothelial glycocalyx degradation, stimulating interest in the endotheliopathy of ACOT. Additional evidence has implicated the innate immune response in general [67], and neutrophils specifically [68] in the pathogenesis of ACOT.

Simultaneous with these provocative studies in civilian trauma centers, the military recognized coagulopathy as the most common source of preventable death in soldiers in the war in Iraq [69]. When confronted with this challenge, the US Army suggested the best solution was to replace the acute blood loss with a blood component formula that would replicate the whole blood lost, thus the genesis of the 1:1:1 concept [70]. In 2007, Borgman et al. [71] reported the US military experience in Iraq suggesting a survival benefit for soldiers resuscitated with an FFP:RBC ratio approaching 1:1 when they required a massive transfusion (10 units of red blood cells (RBC) in 24 h). This report was extrapolated to support the proposed "damage control resuscitation" concept with 1:1:1 as the centerpiece. Although the relative simplicity of this recommendation is appealing, this concept is not intuitive biologically and has prompted vigorous debate that continues today [72–75]. Ultimately these debates stimulated the National Institutes of Health (NIH) to conduct a Trans-Agency Coagulopathy in Trauma Workshop, held in Bethesda in April 2010. Out of this meeting came the consensus that the term "trauma-induced coagulopathy" (TIC) should be employed to describe what was previously referred to as ACOT in order to ensure a common language for research.

Conspicuous among the many questions is whether platelets should be given empirically with the initial administration of FFP and RBC units in patients at risk for TIC. In contrast to platelets for first-line therapy in the United States, the European approach has been to load fibrinogen [76]. Theoretically platelets contribute more to clot strength than fibrinogen, but each component can compensate for deficiencies in the other. A recent randomized study with empiric platelet transfusion for intracranial hemorrhage indicated adverse outcome [77]. The current limitation in assessing platelet function for hemostasis has hampered resolution of this topic [78, 79]. Furthermore is the ongoing debate of the optimal ratio of FFP:RBC units in the patient at risk for TIC. The only randomized trial to date failed to demonstrate a prolonged survival advantage of a 1:1 versus 1:2 FFP:RBC ratio when delivered with platelets [80]. Of note, this randomized trial showed improved survival at 3 h, but statistical difference was lost at 24 h as well as the FDA standard of mortality at 30 days due to attrition in both study groups. The controversy has prompted another NIH workshop to define optimal endpoints for clinical hemostasis research, and it appears a 3–6 h endpoint may be acceptable in certain studies [81]. Independent of the optimal ratio debate, the concept of plasma first resuscitation was extended to prehospital studies, suggesting patients with extended transport times may benefit from plasma in the field [82, 83].

The role of systemic fibrinolysis in TIC has added another layer of controversy. The role of dysregulated fibrinolysis was largely overlooked until the widespread implementation of global viscoelastic hemostatic assays in trauma care, such as thrombelastography (TEG) and thromboelastometry (ROTEM) [84–87]. The CRASH-2 trial reported in 2010 [88] prompted unbridled use of tranexamic acid (TXA) until the limitations of this study were acknowledged [89, 90]. Consequently, it was generally accepted in the United States that TXA should be reserved for selected populations until randomized trials clarify the indications. The most recent randomized trial of prehospital TXA for TBI reported a survival benefit of 2 gm, but without viscoelastic evidence of inhibited fibrinolysis suggesting the benefit may be derived from anti-inflammatory effects (personal communication Martin Schreiber MD). The elucidation of fibrinolysis shutdown [91–93] and subtypes [94] with an associated risk of thromboembolism has added to

the concern of routine TXA administration. Furthermore, the issue of whether goal-directed therapy via viscoelastic assays such as TEG or ROTEM is the optimal management for TIC remains debated. A large retrospective study indicated that TEG-driven resuscitation was more effective than 1:1:1 approach [95], and our recent single-institution randomized study [96] indicated that TEG was more effective in guiding a massive transfusion protocol than conventional laboratory tests (PT/INR, aPTT, platelet count, and d -dimers). In 2013, driven by these ongoing controversies, the NIH funded a Trans-Agency Research Consortium for Trauma-Induced Coagulopathy (TACTIC) in collaboration with the Department of Defense (DOD) with the aim of elucidating the underlying mechanisms of TIC from "road to rehabilitation" [97].

Interestingly, the most recent chapter in hemostasis management of the severely injured is the return to stored low titer O negative whole blood (LTOWB) as the initial resuscitation fluid. Stimulated by military success of the walking donor policy with fresh whole blood [98], the current trend is to administer LTOWB in the field [99] as well as the emergency department [100] back to the future.

In sum, the need to define the scientific basis for blood component administration and regulation of fibrinolysis in the critically injured patient is as clear today as it was 60 years ago and, as optimistically articulated by Mario Stefanini [6], we are making substantive progress. "While the multiplicity of hypotheses and the conflict of experimental findings still deny us a firm theoretical basis for the interpretation of the mechanisms of hemostasis, the impact of the advances of the last 10 years on the diagnosis and management of the bleeding patient has been staggering. New diagnostic tests have greatly increased the accuracy of the diagnosis; broader interest in the isolation of coagulation factors and of platelets points to more specific methods of treatment in the near future. One feels that, with the unending ferment of ideas and fervor of investigation in this field, great progress lies ahead."

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2 Epidemiology of Hemorrhage-Related Mortality

Angela Sauaia, Ernest E. Moore, Charles E. Wade, and John B. Holcomb

Frequency

Uncontrolled bleeding has been reported to cause at least one quarter of all injury-related deaths [1–9], and over 40% of potentially preventable postinjury deaths, both in military [10, 11] and civilian settings [3, 12–14]. A review of preventable and potentially preventable deaths in an urban trauma center from 1998 to 2005 in Los Angeles, California, documented that 40% were due to hemorrhage [15]. A 2019 systematic literature review of pre-hospital deaths due to trauma indicated that exsanguination was responsible for 54–81% of trauma deaths deemed potentially or definitely preventable in studies conducted throughout the world from 2000 to 2013 [16]. The reports on the causes of postinjury death differ widely depending on the data source and definitions. Studies using civilian hospital, state, or national trauma registries (e.g., the American College of Surgeons sponsored National Trauma Data Bank, NTDB[1]) do not include deaths prior to admission, which still represent for over half of the trauma fatalities [17]. Similarly, military patients killed in action (i.e., before arriving at a medical treatment facility) are sometimes not included [11, 18]. It is important that the readers verify these different ways to represent the data (e.g., in-hospital, population-based) when appraising articles.

In a 2011 study by investigators at the United States Army Institute of Surgical Research (USAISR) of combat fatalities occurring in the deployed environment from October 2001 to June 2011, 87% occurred before arrival at a medical treatment facility [10]. Of these, 24% were considered potentially survivable, and were predominantly (91%) due to hemorrhage. During the same period, the USAISR group reported that only 4.6% of all combat deaths occurred after reaching a military treatment facility (a.k.a, died-of-wounds), of whom close to half were deemed potentially survivable, again largely due to acute hemorrhage (80%) [19].

In contrast to military literature, few studies have assessed the role of hemorrhage in civilian trauma deaths occurring in the pre-hospital setting, due to difficulties in obtaining granular

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[1]Available at https://www.facs.org/quality-programs/trauma/tqp/center-programs/ntdb, accessed 11/30/2019.

data [20–22]. The table shows the impact of hemorrhage in pre-hospital (when available) and in-hospital deaths in several epidemiological investigations listed in chronological order of the period covered by the study. A civilian 1992 epidemiologic study in Denver City and County, Colorado, found that 34% of the deaths occurred in the pre-hospital setting, and of these, over one third were due to exsanguination [3]. Overall, the study determined that 31% of all deaths attributed exclusively to bleeding (i.e., excluding those in combination with traumatic brain injury (TBI)) occurred in the pre-hospital phase. A similar study in San Diego, California, also in 1992, in which autopsy data were available for all fatalities, also found that 40% of the fatalities happened before arrival of pre-hospital providers, and that close to a third of all deaths were due to uncontrolled hemorrhage in the chest, abdomen, or both cavities [5]. Unfortunately, this study did not specify the impact of hemorrhage specifically in the pre-hospital setting.

Stewart et al. [23] studied in-hospital trauma fatalities from 1995 to 2001 occurring at one of three level 1 trauma centers in San Antonio, Texas, and attributed shock as the cause of death in 21% of the cases. Almost two decades later, a study of autopsies of individuals who died of trauma pre-hospital in the field in Miami-Dade county in 2011 [12] reported that hemorrhage caused a similar percentage of the deaths (34%), second only to neurotrauma (36%); combined hemorrhage and neurotrauma caused 15% of the deaths. Close to a third (29%) of all deaths were judged to be potentially survivable, due primarily (54%) or partially (10%) to hemorrhage. In a study in Harris County, Texas, Drake et al. [14] combined 2014 data from autopsy, medico-legal death investigation reports, emergency medical service, hospital records, hospital trauma morbidity and mortality reports, in a comprehensive epidemiological investigation of trauma deaths. They determined that close to half (46%) of the fatalities still happened in the pre-hospital phase, 36% in the index-hospitalization, and 18% after the index-hospitalization. Hemorrhage was implicated in 20% of the deaths in the pre-hospital phase and 20% of the in-hospital deaths. These investigators also demonstrated that hemorrhage was most impactful in deaths that were deemed preventable/potentially preventable (P/PP): hemorrhage caused 55% of the P/PP deaths in the pre-hospital phase, and 28% of the in-hospital P/PP deaths. A prospective, multicenter Western Trauma Association study of trauma deaths between December 2015 and August 2017 in 18 trauma centers across the United States, published in 2019, reported exsanguination caused 23% of all deaths among patients who were transported to trauma centers, second to TBI (45%) and followed by physiologic collapse (25%) [6]. Notably, over half (58%) of all bleeding-related deaths occurred during transport or in the emergency department. Collectively, these studies demonstrate the substantial impact of hemorrhage in trauma deaths in general, and among deaths at the scene and P/PP deaths specifically. They highlight the need for improved resuscitation strategies in the pre-hospital arena as well as better data monitoring of this phase in trauma care.

Most studies outside the United States reveal a similar high impact of hemorrhage in trauma deaths. A 2005 population-based investigation in Newcastle, Australia, attributed bleeding as the cause of 33% of all deaths (both pre- and in-hospital) [7]. In Canada, where blunt mechanism predominates, a study of deaths occurring in a level 1 trauma center from 1999 to 2003 implicated hemorrhage in 15% of all in-hospital deaths, of which 16% (mostly due to blunt pelvic injury) were judged to be preventable [24]. In Stavenger, Norway, where the autopsy rate exceeds 95%, 25% of the trauma deaths from 1996 to 2004 were due to exsanguination, half of which occurred within 1 hour postinjury [8]. In a population-based study in Berlin [25], of 440 trauma deaths during 2010, 10% were attributed to exsanguination, which was defined very narrowly as: "*coincident/singular blunt and/or penetrating severe injury/ies to various organ/s or organ systems, which were primarily lethal due to hemorrhagic shock without destruction/dysfunctions of vital structures as the leading cause of death.*" Of the remainder, close to half were ascribed to "polytrauma" and 38% to TBI. It would be plausible to assume that a substantial proportion of the so-called "polytrauma" deaths were associated with hemorrhage. Sixty percent of the deaths in the Berlin study occurred at the scene,

of which 14% were attributed to exsanguination, and 24% of all exsanguination-related deaths occurred at the scene. Another Canadian study in the Foothills Medical Centre in Calgary analyzed 1000 consecutive in-hospital trauma deaths among 9941 patients admitted from 2005 to 2013, of which 27% were attributed to exsanguination [26]. In a hospital-based study in Turkey, from 2010 to 2013, 22% were attributed to circulatory collapse and another 11% to circulatory collapse plus TBI [27]. In Brazil, hemorrhage claimed 18% of the trauma deaths in an urban hospital. In an assessment of in-hospital deaths in a Netherlands urban trauma center reported that exsanguination caused 9% of the 2007–2012 fatalities and only 3% in 2013–2016 [28]. These were the periods before and after the implementation of hemostatic resuscitation and damage control procedures in their hospital. TBI was the main cause of death in both periods (58% in 2007–2012 and 76% in 2013–2016). The definition of exsanguination was not specified in the article, but a number of death causes potentially related to hemorrhage were listed (e.g., chest injury, cardiac arrest). A number of reasons may explain the different proportion of hemorrhage in non-US versus US studies including (but not limited to) higher frequency of penetrating trauma in the United States versus other countries; different definitions of hemorrhagic and TBI deaths and disparities in injury prevention (e.g., alcohol-related injuries, road conditions, trauma systems, emergency medical services, availability of resuscitation-related resources).

Temporal Trends

Temporal trends in cause-specific mortality proportions were evaluated at the Scripps Mercy Hospital, in San Diego, California, US from 2000 to 2011, finding no significant change in the proportion attributed to acute hemorrhagic shock, which remained slightly over one quarter of all deaths [4]. In an urban trauma center in Texas, a comparison of the proportion of deaths due to hemorrhage before (2005–2006) and after (2012–2013) the implementation of a bleeding-control bundle of care showed an unadjusted decrease from 36% to 25% [9]. Specifically among early in-hospital deaths (<1 hour postinjury), there was a reduction in the proportion of hemorrhage as the primary cause from 60% in 2005–2006 to 38% in 2012–2013. The authors credited the improvement to the implementation of a multi-modal bleeding control bundle encompassing: (1) accurate identification of the bleeding patient; (2) pre-hospital and hospital damage control resuscitation: (3) pre-hospital and hospital use of hemostatic techniques such as extremity and junctional tourniquets, pelvic binders, and hemostatic dressings; (4) resuscitative endovascular balloon occlusion of the aorta; (5) coagulation monitoring with thrombelastography; (6) tranexamic acid for significant fibrinolysis; (7) decreased time to operating room and interventional radiology; and (8) goal-directed resuscitation with blood products as bleeding slows. A subsequent study using the same dataset showed that among potentially preventable in-hospital deaths, hemorrhage remained frequent (2005–2006: 48% vs. 2012–2013: 43%, $p = 0.55$) [29].

The study of the epidemiology of hemorrhagic deaths requires focused attention to the denominator used in the report or study. As the above-cited investigations demonstrate, a substantial proportion of the deaths occurring pre-hospital, both in civilian and military settings, are unequivocally non-preventable, for which primary prevention may be the only solution. Of course, the judgment of whether a death is preventable or non-preventable may involve some degree of subjectivity. In non-obvious cases, it is advisable to apply more objective criteria to estimate the probability of survival, such as the TRISS [30, 31] (Trauma and Injury Severity Score) probability of death (e.g., classify as non-preventable deaths of injured patients with <10% TRISS probability of survival), or similar model. Removing the non-preventable deaths from the denominator, and concentrating only on the subset of PP/P deaths, highlights the group who may benefit the most from focused interventions.

Mechanisms

In the recent Western Trauma Association study, attending providers of 18 US trauma centers adjudicated the cause of death (COD) immedi-

ately following the patient's demise using standardized definitions (with autopsy confirmation, when available) from December 2015 to August 2017. Their data revealed that blunt patients were more likely to have TBI (47.8% vs. 37.4%, $p < 0.0001$) as the primary COD while for penetrating patients, exsanguination was the more frequent primary COD (51.7% vs. 12.5%, $p < 0.0001$). The above-mentioned Canadian study, where penetrating trauma is relatively infrequent, 48% of the hemorrhagic deaths (i.e., in-hospital deaths) were due to blunt injuries, and 52% were from a penetrating mechanism [24].

We also queried the Trauma Activation Protocol (TAP) database of the Ernest E Moore Shock Trauma Center at Denver Health (a mature, urban, Level I trauma center) enrolled by professional research assistants onsite 24/7. This database started in 2014 and up to December of 2018 included 907 consecutive injured adults (age >= 18 years) meeting criteria for the highest level of activation (*any* of the following: (1) GCS < 8 with presumed thoracic, abdominal, or pelvic injury, (2) respiratory compromise, obstruction, and/or intubation with presumed thoracic, abdominal, or pelvic injury, (3) blunt trauma with SBP < 90 mm Hg, (4) mechanically unstable pelvic injury, (5) penetrating injuries with injury to neck and/or torso with SBP < 90, mm Hg, gunshot wound penetrating the neck/torso or stab wounds to the neck/torso that require endotracheal intubation, (6) amputation proximal to the ankle or wrist, or (7) the emergency medicine attending or chief surgical resident suspects the patient is likely to require urgent operative intervention). Exclusion criteria are: initial blood sample was not collected within 1 hour of injury, transfers from external hospitals, documented chronic liver disease (total bilirubin >2.0 mg/dL) or advanced cirrhosis discovered on laparotomy, known inherited defects of coagulation function (e.g., hemophilia or Von Willebrand's disease), subsequent downgrades from trauma activation to trauma alert to non-trauma status in the emergency department, pregnancy or prisoner status. All TAP patients have field or hospital arrival blood samples obtained to conduct viscoelastic coagulation tests.

All deaths in the TAP database are adjudicated using clinical, and autopsy (when available) results in weekly meetings with a multidisciplinary team and categorized as due to hemorrhage, TBI, refractory shock, organ failure, and other causes (which includes multiple causes, e.g., TBI and hemorrhage). In our TAP database (data not published), with an overall mortality of 13% (119/907), hemorrhage accounted for 21% of all deaths. Among non-survivors with hypotension (systolic blood pressure < 90 mmHg) in the field, hemorrhage was responsible for 34% (20/59) of the deaths (Fig. 2.1). Overall, 64% (16/25) of the hemorrhagic deaths were caused by penetrating injuries (70% among hypotensive patients), compared to 22% (12/54) of the traumatic brain injury deaths (31% in hypotensive patients). Of the early deaths (<24 hours postinjury), 36% (25/69) were due to hemorrhage, of which 16% were deemed PP/P and these proportions were similar in hypotensive patients. Among non-hemorrhagic deaths, a similar proportion (89%) was judged non-preventable. A little over half of the hemorrhagic deaths were accompanied by abnormal viscoelastic tests suggesting laboratorial trauma-induced coagulopathy (TIC). Penetrating trauma was the predominant mechanism in hemorrhagic deaths, regardless of hypotension in the field (64% in all 119 non-survivors; 70% in the 59 hypotensive non-survivors). Similarly, penetrating trauma predominated among refractory shock deaths (67% regardless of hypotension in the field). Conversely, TBI and organ failure deaths were mostly due to blunt injuries (78% and 67%, respectively). Deaths due to multiple causes (which often involve hemorrhage) or causes other than the above-named were mostly due to blunt trauma (68%).

The incidence of blunt versus penetrating injuries in the catchment area of a trauma center may have implications for trials testing hemostatic interventions, as trauma centers with a predominance of penetrating injuries may be more likely to observe an effect than a center with higher proportion of blunt trauma [32]. Yet, as shown in Table 2.1, a crude inspection of civilian epidemiological studies suggest the proportion of

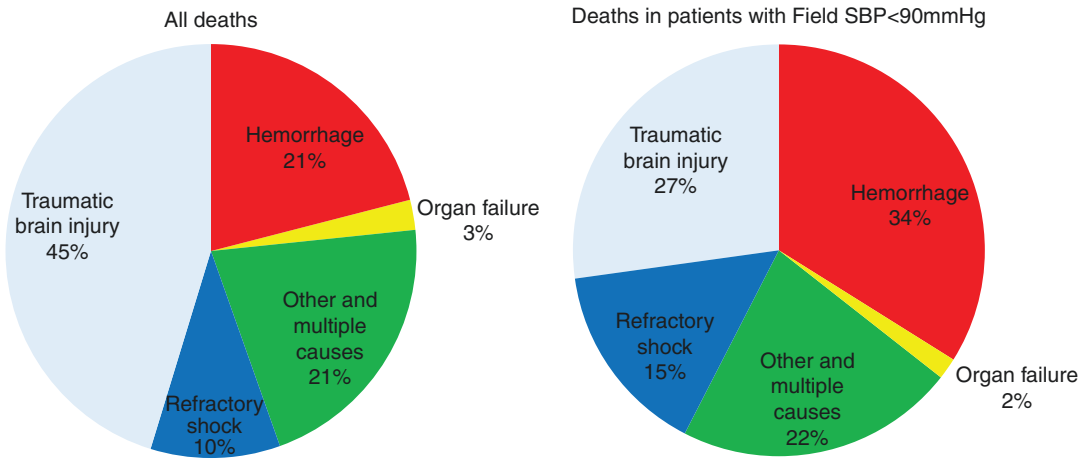


Fig. 2.1 Cause of death distribution in the Trauma Activation Protocol database, which includes injured adults (age >= 18 years) meeting criteria for the highest level of activation at the Ernest E Moore Shock Trauma Center at Denver Health from 2014 to 2018, (n = 907)

Table 2.1 Selected civilian studies addressing hemorrhagic deaths

Studies	Year(s) covered by study	Setting	% blunt trauma	% of pre-hospital deaths due to hemorrhage	% of in-hospital deaths due to hemorrhage
Sauaia et al. [3]	1992	Population-based Denver, CO	51%	36%	41%
Shackford et al. [5]	1992	Population-based S. Diego, CA	71%	31%	
Stewart et al. [23]	1995–2001	Hospital-based S. Antonio, TX	71%	NA	21%[a]
Tien et al. [24]	1999–2003	Hospital-based Toronto, Canada	87%	NA	15%
Evans et al. [7]	2005	Population-based Newcastle, Australia	86%	33%	
Consunji et al. [52]	2004–2007	Hospital-based Manila, Philippines	41%	NA	28%
Soreide et al. [8]	2010	Population-based Stavenger, Norway	87%	12.5%[c]	12.5%[c]
Kahl et al. [4]	2000–2011	Hospital-based S. Diego, CA	69%	NA	28%
Kleber et al. [25]	2010	Population-based Berlin, Germany	87%	14%[d]	4%[d]
Trajano et al. [73]	1995, 2000, 2005, 2010	Hospital-based Campinas, Brazil	73%	NA	18%
Davis et al. [12]	2011	Population-based Miami-Dade County, FL	53%	34%	NA
Roberts et al. [26]	2005–2013	Hospital-based Calgary, Canada	94%	NA	27%
Arslan et al. [27]	2010–2013	Hospital-based Ankara, Turkey	85%	28%[e]	29%
Oyeniyi et al. [9]	2005–2006 and 2012–2013	Hospital-based Houston, TX	81–80%	NA	2005–2006: 36% 2007–2013: 25%

(continued)

Table 2.1 (continued)

Studies	Year(s) covered by study	Setting	% blunt trauma	% of pre-hospital deaths due to hemorrhage	% of in-hospital deaths due to hemorrhage
Drake et al. [14][b]	2014	Population-based Harris County, TX	62%	20%	20%
Jochems et al. [28]	2007–2016	Hospital-based Utrecht, Netherlands	98%	NA	2007–2012: 9% 2013–2016: 3%
Callcut et al. [6]	2015–2017	Hospital-based 18 centers across US	73%	NA	23%

[a]Attributed to shock
[b]18% of the trauma-related deaths occurred after the index hospitalization, of which 5 (1%) were attributed to hemorrhage
[c]Authors estimation based on the time from injury to death reported in original study
[d]Definition excludes "polytrauma deaths"
[e]Death at scene or during transport but transported to the hospital

death attributable to hemorrhage does not seem to vary substantially according to the distribution of blunt (versus penetrating) mechanism. Of course, time from injury to bleeding control significantly confounds and/or modifies the effect of blunt vs penetrating mechanism. Blunt trauma may require prolonged extrication and result in multiple bleeding sites, while victims of penetrating injuries who reach medical attention early postinjury more often (but not always) have less bleeding sites allowing faster hemostasis. Notably, while penetrating injuries are less common in most places, thus a less frequent cause of postinjury fatalities than blunt injuries, they account for a large percentage of massively transfused patients [33–35].

Timing of Hemorrhagic Death

Understanding the timing of hemorrhagic deaths is crucial to determine when hemostatic therapies can be most effective, and which outcomes (i.e., death, transfusions, non-bleeding complications such as thrombotic events) can be affected by such therapies [32, 36]. These therapies focus on maintaining physiology in the pre-hospital as well as in the initial hospital setting, optimizing resuscitation and hemostasis while mechanical control of bleeding is achieved. As civilian and combat epidemiological investigations demonstrate, trauma deaths occurring within minutes of injury are mostly due to irreparable, devastating injuries, such as catastrophic or total body disruption, lacerations of the brain, brainstem, spinal cord, or freely ruptured major vessels [1, 3, 12, 13, 15, 17–19, 24, 37–42]. The second group includes patients who die over the ensuing hours mostly from hemorrhage and severe TBI. It is this second group of hemorrhagic deaths group that is most amenable to hemostatic interventions. Overwhelming evidence from randomized controlled trials (RCTs) [42–50] and large observational studies [51] assessing a variety of hemostatic and resuscitation therapies show that the vast majority of the hemorrhagic deaths occur within 24 hours, and peak within 6 hours postinjury. The above-mentioned Canadian study of 1000 in-hospital trauma deaths demonstrated that 38% of the in-hospital exsanguination deaths occurred within 6 hours post admission; 30% within 6–24 hours (total of 68% within 24 hours), 24% within 24–48 hours, and 8% after 48 hours [26]. In two hospital-based reports, Arslan and colleagues [27] in Turkey reported that 97% of the deaths due to circulatory collapse occurred within 24 hours, while Consunji et al. [52] in the Philippines observed that 69% of exsanguinations deaths occurred within 24 hours. Patients with non-compressible torso trauma hemorrhage appear to have even more rapid deaths, as shown in a 2012–2014 analysis of NTDB data (median time from injury to death varying from 28 to 40 minutes depending on mechanism) [53].

The assessment of two Resuscitation Outcomes Consortium (the first addressing traumatic hypovolemic shock [48], and the second addressing TBI [54]) trials by Tisherman and colleagues [42] showed that 82% of the in-hospital deaths in the shock cohort occurred within 24 hours (median time 2 hours when shock was not associated with TBI, and 4 hours when combined with TBI). Although not clearly stated in this assessment, the time "zero" seemed to be the time of injury.

The distribution of deaths over time after injury in the above-described TAP in-hospital database is summarized in Fig. 2.2. As expected hemorrhagic deaths were more frequent in the first 6 hours after injury and absent after 24 hours. It is important to note that the ROC trials tested pre-hospital interventions (hypertonic saline) but reported time zero as the time from emergency department admission, while the TAP database "starts the clock" at time of injury.

A 2017 review of deaths in trauma-focused RCTs showed that median time to hemorrhagic death, in trauma systems with rapid transport from injury to hospital, varied from 2.0 to 2.6 hours from admission [36]. Yet, the studies included in the review varied or were unclear about when they "started the clock," with some starting it at time of injury (e.g., Polyheme study [50]) while others started at the emergency department admission (e.g., ROC trials [48]). The survival curve of the Prehospital Air Medical Plasma (PAMPer) trial [45] showed an early separation of the two groups starting 3 hours after randomization, and persisting until 30 days ($p = 0.02$). In the large CRASH2 trial, representing dominantly developing countries, 34% of all deaths were attributed to bleeding, 50% of which occurred <10 hours, and 60% in <24 hours post-randomization (by design, randomization happened in the hospital for patients admitted within 8 hours postinjury) [32, 47].

An analysis of the PROPPR randomized controlled trial, which assessed the effect of 1:1:1 ratios of red blood cells: plasma: platelets transfusions compared to 1:1:2 ratios, showed that at 3 and 6-hours post-randomization, hemorrhage accounted for over 90% of all deaths

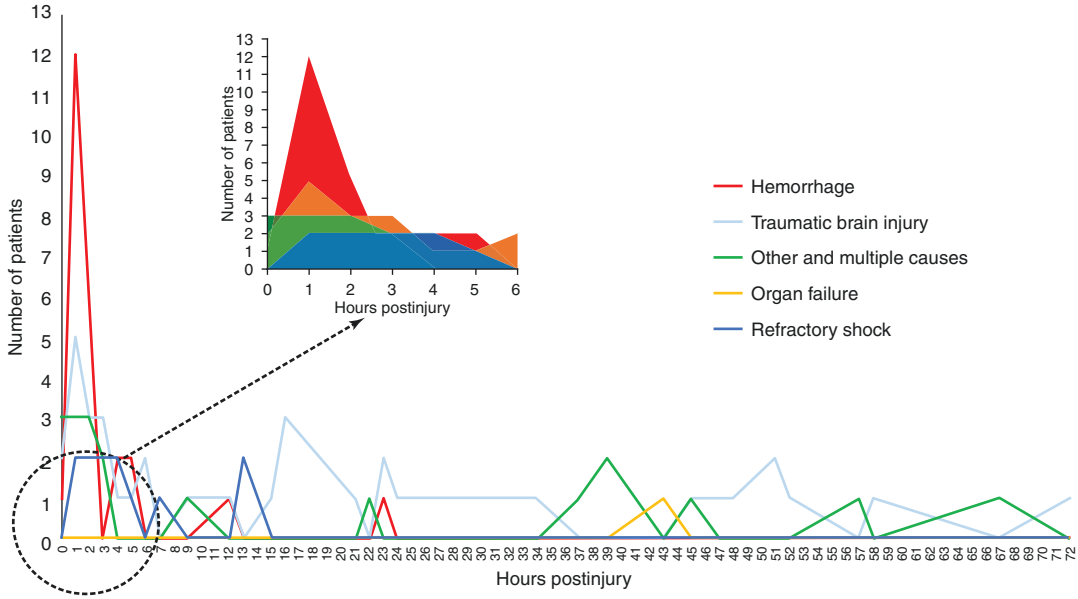


Fig. 2.2 Temporal distribution of deaths in the Trauma Activation Protocol database, which includes injured adults (age >= 18 years) meeting criteria for the highest level of activation at the Ernest E Moore Shock Trauma Center at Denver Health from 2014 to 2018, ($n = 907$)

[36]. The PROPPR study included activation patients who were deemed at risk for massive transfusion and excluded obviously fatal TBI [43]. From admission, the median time to hemorrhagic death was 2.4 hours (Interquartile range, IQR: 1.2–4.0) [36].

Using the above-mentioned PROPPR analysis as a template, we examined the above-described TAP database, including only patients who were hypotensive in the field (systolic blood pressure < 90 mmHg), is shown in Fig. 2.3. The panels in Fig. 2.3 show cause-specific, Kaplan-Meier failure curves for all-cause mortality, and cause-specific deaths occurring within 3 hours postinjury (Panel A, 180 minutes), 6 hours (Panel B, 360 minutes), 24 hours (Panel C), and 600 hours (Panel D). For the cause-specific curves, deaths due to other causes were censored. These data demonstrate that close to half of all deaths in the first 3 and 6 hours were due to hemorrhage, while 20% were due to other or multiple causes (multiple causes often involve hemorrhage), and 13–16% (at 3 and 6 hours, respectively) were due to refractory shock, often following hemorrhage. The median time from injury to hemorrhagic death was 1.9 hours (IQR: 0.9–3.9). During the same time periods, close to 20% were due to TBI. The disparity between PROPPR and TAP estimates is likely due to several differences: (1) entry criteria (PROPPR enrolled transfused patients while for the above analysis of TAP we included all hypotensive

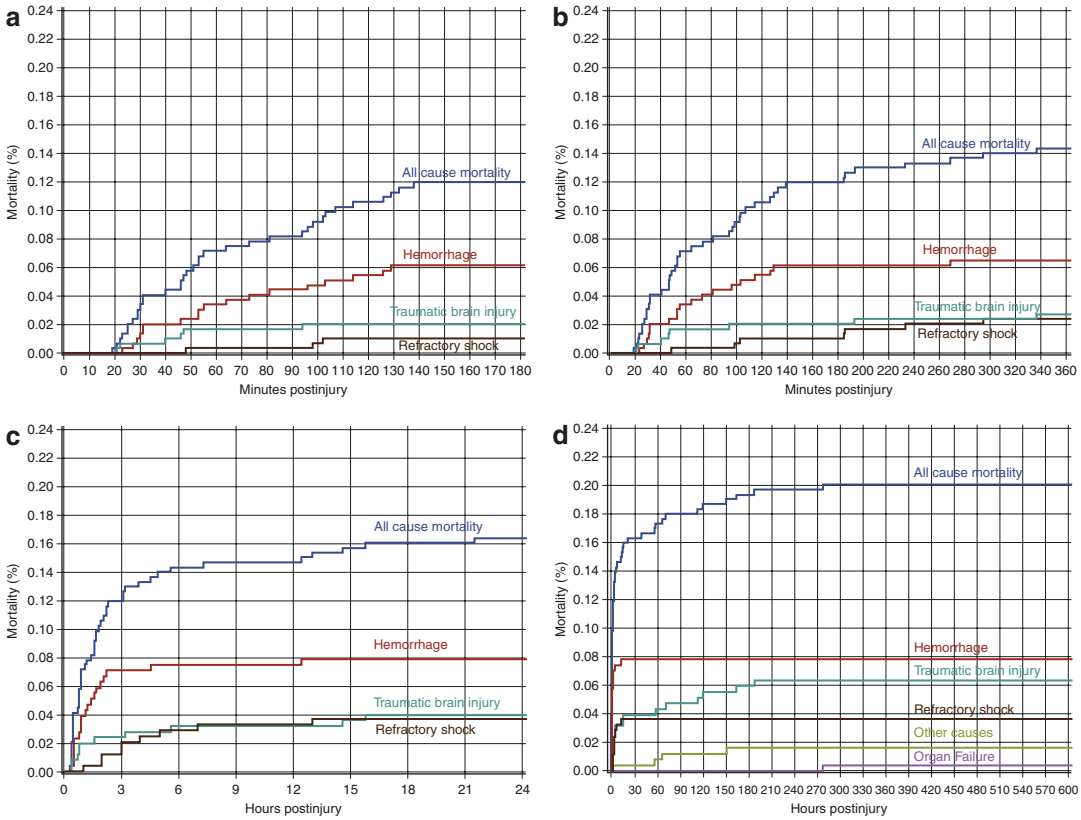


Fig. 2.3 Mortality (%) stratified by cause of death in the Trauma Activation Protocol (TAP) study in patients with hypotension (field systolic blood pressure < 90 mmHg) meeting activation criteria at the Ernest E Moore Shock Trauma Center from 2014 to 2018: (**a**): deaths within first 180 minutes (3 hours); (**b**): deaths within 360 minutes (6 hours); (**c**): deaths within 24 hours; (**d**): deaths within 600 hours (all in-hospital deaths). Other causes include deaths due to multiple causes

patients, of whom 58% required transfusions; (2) "time zero" (TAP uses injury time, while PROPPR uses randomization time, which occurred within approximately 20 minutes of hospital arrival), and (3) death categories, with TAP having two additional groups (refractory shock and other/multiple causes).

Definition of Hemorrhagic Deaths

Traditionally, the section addressing "definition" is presented first, yet, we found that it was important to provide first the evidence upon which we can base the definition. Most trauma surgeons would have little difficulty defining hemorrhagic death on the spot, especially in the absence of TBI. Defining it retrospectively or in the presence of concomitant TBI, however, is a challenge, as the TBI diagnosis requires imaging (not available in patients who died rapidly) or autopsy (not always available). Ultimately, hemorrhagic deaths are due to three main reasons, as follows:

1. Exsanguination because mechanical control of bleeding was not achieved, either because the time between injury and medical attention was too long, the injury was too severe (e.g., complete transection of large blood vessel), or there were multiple significant bleeding sites.
2. Refractory shock due to physiologic exhaustion, eloquently described by Samuel Gross in 1872 as the "rude unhinging of the machinery of life," occurs when mechanical bleeding is controlled and volume is replaced but resuscitation is still unsuccessful in returning cardiovascular homeostasis, likely due to irreversible mitochondrial injury.
3. Trauma-induced coagulopathy (TIC), when mechanical bleeding is controlled and volume is replaced but disseminated insidious bleeding persists due to dysfunctional clotting [34, 35, 55].

In the above-described TAP database, all patients are prospectively classified according to the TACTIC[2] clinical coagulopathy score (CCS) [56], which grades TIC as

- Grade I: Normal hemostasis (negative)
- Grade II: Mild coagulopathy, no intervention required except direct pressure or temporary gauze tamponade (equivocal)
- Grade III: Coagulopathy refractory to direct pressure, requiring advanced hemostasis techniques (e.g., electrocautery, topic hemostatic agents, staples, or suturing) (possible positive)
- Grade IV: Coagulopathy requiring adjunctive blood component therapy or systemic therapeutics in response to continued bleeding despite above surgical hemostatic maneuvers (positive)
- Grade V: Diffuse persistent bleeding from multiple sites remote from injury; e.g., endotracheal tube, intravenous catheter, chest tubes, etc. (definitive positive)

In TAP, 56% (14/25) of the patients who died of hemorrhage had severe CCS (Grades IV or V) in the emergency department (ED), 44% in the operating room (OR), and 20% in the intensive care unit (ICU).[3] These results suggest that severe TIC is common upon hospital presentation among patients who eventually died of hemorrhage, although it appears to be much less frequent later in the ICU. In contrast, the interesting Prehospital Resuscitation on Helicopters Study (PROHS) multicenter study [57], which focused on helicopter-transported patients who survived longer than 30 minutes after hospital arrival during 2015, showed that only 20% of the patients whose death was deemed due to hemorrhage had clinical coagulopathy, defined as "*surgeon-confirmed bleeding from uninjured sites or injured sites not controllable by sutures, and requirement of active resuscitation,*", similar to

[2]TACTIC (Trans-Agency Consortium for Trauma-Induced Coagulopathy) represents a consortium of recognized experts who have partnered in a collaborative effort under the auspices of the National Institutes of Health and the Department of Defense to investigate coagulopathy after trauma.

[3]Unpublished data.

the TACTIC-CCS grades IV and V. It is conceivable that differences in the location where TIC was assessed (ED, OR, ICU) may be responsible for the disparities.

Definitions based on the amount of blood/fluids transfused suffer from survival bias (i.e., patients need to be alive to receive large volumes of blood or fluid) and are based on existing resources or practices (i.e., availability of blood, blood transfusion protocols). Postinjury estimated blood loss (EBL) is largely imprecise, visual estimation by the attending providers onsite, supported by more objective measurements of intra-operative blood loss (e.g., difference between dry/wet weight of blood soaked items) [58, 59]. Often, there is poor (if any) information on blood loss at the site of injury or during transport. Brecher and colleagues [60] proposed in 1997 a simple mathematical model for surgical blood loss based on the estimated total blood volume; initial (i.e., at the start of the operative procedure) and minimum hematocrit allowable, i.e. the transfusion trigger; and the number of red blood cell units transfused. However, in trauma the initial hematocrit is unknown, although in relative young healthy patients, one could assumed it normal. Frank et al. [61], in 2010, produced a simulation of trauma scenarios, and observed that estimation based on the visual scenario and vital signs led to overestimation of small actual volumes, and underestimation of higher volumes, regardless of whether the "patients" were hemodynamically stable or unstable. Several studies confirmed the inability of healthcare providers of different backgrounds to accurately [58, 59, 62]. The MAR method, created by Merlin and colleagues [63] in 2009, uses a fist to cover a surface area of blood that equals 20 ml, and allegedly requires only a brief 1-minute instruction.

Time to hemostasis, defined as no intraoperative bleeding requiring intervention in the surgical field (OR) or resolution of contrast blush on interventional radiology (IR), has been recently tested as an outcome by Chang et al., in a subgroup of patients enrolled in the PROPPR trial [43] who required emergent OR/IR hemostatic intervention [64]. These authors reported that few patients died over 72 hours if hemostasis was obtained, and, after adjustment, every additional 15 minutes was associated with increased complications and death. However, as acknowledged by these investigators, this outcome is subject to survival bias as patients who did not achieve hemostasis or exsanguinated prior to OR/IR procedures had to be excluded from the analysis. Other limitations include: (1) only patients for whom OR or IR is performed are eligible; (2) this outcome is not obtainable retrospectively; and (3) there is considerable subjectivity and inter-practice variation in its assessment.

Alternatively, time-specific death has been proposed as a suitable substitute to cause-specific death, as different causes of death are consistently shown to occur at different times, with hemorrhagic deaths usually restricted to less than 24 hours, and peaking within 6 hours, as described above [32, 36]. Collectively, the evidence supports the adoption of earlier endpoints for RCTs testing hemostatic therapies in trauma [32, 36]. Indeed, at a 2019 workshop convening international experts in trauma hemostasis sponsored by the National Institutes of Health, a recommendation was made to use early endpoints, i.e., within 3–24 hours, as outcomes in hemostatic trials. In addition, it is essential to clearly identify the starting time-point (i.e., times of injury, emergency medical services (EMS) dispatch, EMS arrival, hospital admission, randomization, intervention initiation, etc.), and, in addition, document all the other injury and injury-care related times to allow comparisons with other studies that may have used other start times.

Earlier endpoints in RCTs of hemostatic interventions must continue to be accompanied by the assessment of later complications (infections, ventilation time, multiple organ failure, acute distress respiratory syndrome, thromboembolism, etc.) or any outcome requiring long enough survival to be experienced it (e.g., 24-hour transfusions). While complex, it is imperative that we evaluate the later effects of the early hemostatic interventions, as some of them may pose an increased risk of thrombosis (e.g., antifibrinolytics) [65–67]. The main reason for the complexity of such evaluations is related to a

potential differential early survival of patients in these trials. If the experimental treatment tested in the RCT is indeed superior, patients surviving due to this beneficial effect will subsequently confront a higher risk for later complications. Because their counterparts allocated to the inferior treatment arm died early, the comparison group is now comprised of survivors with less severe injuries who are less susceptible to later complications. In addition, the intervention that reduced deaths due to bleeding may not affect other conditions, such as central nervous system injuries, prolonged immobility, and ventilation, that predispose the survivors to delayed complications. Consequently, the treatment-control difference in later outcomes for the survivors is now a biased estimate of the impact of the intervention on later outcomes. This is a special case of survivor bias, labeled by statisticians as "truncation-by-death" [68], in which any later outcome is indeterminate among patients who did not live long enough to have the "opportunity" to develop later complications [68, 69]. The example of the ARDSnet (Acute Respiratory Distress Syndrome Network) clinical trial comparing low-tidal volume and traditional ventilation for acute respiratory distress syndrome (ARDS) [70] has been used to discuss this issue [71, 72]. The study found a significant decrease in 180-day mortality favoring the low-volume group (31% vs 40%, $p = 0.003$), but detected no significant differences in intensive care unit stay (ICU, 9.47 vs 9.99, $p = 0.47$) and ventilation days (11.88 vs 12.60, $p = 0.32$). It is conceivable that the survivors of the control group were healthier than the survivors of the experimental (low tidal volume) group, whose survival was due (at least partially) to the new intervention, thus requiring less ICU and ventilation days.[4]

We examined whether this phenomenon was observed in trauma trials. The PROPPR study [43], which randomized patients to plasma, platelets, and red blood cells in a 1:1:1 (experimental) vs a 1:1:2 (control) ratio, observed a 4 percent point decrease in 24-hour mortality (13% vs 17%, $p = 0.12$) and in 24-hr hemorrhagic death (9% vs 15% $p = 0.03$) favoring the experimental intervention, but virtually identical rates of ARDS (14% for both groups). One would have anticipated a higher rate of ARDS in the survivors of intervention group. The PAMPer trial [45], which enrolled air-transported, severely injured patients, offers another possible example: there was a large difference in mortality favoring pre-hospital plasma (experimental) vs standard of care (23% vs. 33%; difference, $p = 0.03$), but the incidence of ARDS in the pre-hospital plasma group was similar to the standard of care (19% vs 21%, $p = 0.50$ unadjusted for multiple comparisons). Once again, one would expect a higher incidence of ARDS in the sicker survivors of the experimental intervention.

If the investigators of a RCT suspect some of their outcomes (e.g., ARDS, venous thromboembolisms, multiple organ failure, infections) may be censored or "truncated-by-death" (TBD), the first step is to define the severity of the TBD problem, that is, how much the early survival affected the comparability of the study groups [69]. For this purpose, we must determine whether and how survivors of the early postinjury period in the experimental and control groups differ (not by statistical testing, rather by using stricter criteria such as standardized mean differences). Of course, this step is only as successful as the measured variables, as there may be hidden confounding from unmeasured factors. Thus, it is essential that researchers anticipate the occurrence of TBD and collect the necessary variables to define severity for this later comparison. This may include scores of disease severity (SOFA, APACHE, etc.), coagulation assays, and serial indicators of physiologic derangement collected at short intervals, etc., which can allow the investigators to determine how comparable the survivors of the initial insult were. If the TBD interference is minimal, we can be more confident in the crude difference in later outcomes. Conversely, if TBD is of concern, then we may proceed to remediation. It is possible to estimate

[4] Just for the sake of the example, we did not account here for the criticism that the tidal volume in the control group of this study may have been set too high, thus increasing the probability of success of the experimental treatment [74].

what statisticians labeled the "survivor average causal effect" (SACE, the average effect in people who would have survived no matter which treatment they were allocated to) for later outcomes. There are a variety of statistical approaches to estimate the SACE (sensitivity analyses, propensity score-based methods, principal strata effect) [69]. Most approaches to estimate the SACE are complex, but Chiba and Vanderweele [72], using ARDSnet data, proposed a simple method, which can be easily implemented. A simplified illustration follows: a hypothetical study on an intervention for traumatic hemorrhage shows mortalities of 5% in the intervention group vs 12% in the control group, resulting in a statistically significant ($p < 0.05$) absolute difference of 7%. The authors of the study also compared the incidence of ARDS in the survivors of the two groups, and observed 14% in the intervention group and 12% in the control group (absolute difference 2%, $p > 0.05$). Although not significant, this difference could raise concerns about potentially harmful effects of the intervention under study. However, the survivors in the control group were healthier than the survivors of the intervention group. If certain assumptions are satisfied,[5] the Chiba-Vanderweele method to estimate the SACE "adjusts" the observed absolute difference of 2% by subtracting a "sensitivity parameter." This sensitivity parameter is the expected difference in ARDS incidence between the "relatively healthy" survivors in the control group and the "not-so-healthy" survivors in the intervention group. This sensitivity parameter is defined by the investigators (or ideally by an independent team of experts). In our hypothetical study, based on historical trends, an independent panel of experts determined that the expected difference in ARDS incidence in survivors with the illness severity observed in the two study groups would be: [intervention ARDS – control ARDS = 5%]. Applying this sensitivity parameter = 5% to the observed difference of 2%, we estimate SACE to be [2–5%] = −3%. We can also provide a range of sensitivity parameters (e.g., 2–8%), resulting in a range of SACE estimates. We can also estimate the 95% confidence interval of the SACE, by simply subtracting the sensitivity parameter from the upper and lower confidence limits of the observed ARDS difference between survivors of the study groups. In common, all the methods to calculate SACE, be they simple or highly sophisticated, require certain assumptions, which need to be verified and expressed carefully. In our hypothetical example above, the definition of a plausible sensitivity parameter demands transparency and careful review of existing evidence on ARDS incidence in populations with varying degrees of severity. After examining the above-described trauma trial examples, it appears that the TBD phenomenon, albeit present to some degree, does not play a large role in this setting.

Using early mortality as an outcome presents an additional advantage related to a basic property of statistical power: for the same difference between two proportions, the statistical power decreases as the two proportions approach 50%. This is related to the degree of variability in the population, which is an essential component of statistical power calculations. A proportion of 50% indicates the maximum variability in a population; it shows more variability than either 20% or 80%, because 20% and 80% indicate that a large majority *do not* or *do*, respectively, have the outcome of interest. Because as times goes on, more deaths occur in both the experimental and control groups, the same absolute difference in percent points that was statistically significant at 3 hours, may lose significance at 12 hours or 30 days. Of course, the experimental treatment may indeed have harmful effects manifested through death due to other causes (e.g., worsening of brain injury) or later complications (e.g., increase of inflammation and organ dysfunction, predisposition to infections); thus monitoring other causes of death is essential to assess the safety of the experimental treatment.

In conclusion, hemorrhage remains the most important cause of preventable deaths following

[5] Assumption 1: Individuals in the Intervention group have LOWER survival than individuals assigned to the Control group (also known as the "monotonicity assumption"); Assumption 2: The survivors in the Control group are healthier than survivors in the Intervention group.

injury both in civilian and military settings. A standard definition of hemorrhagic death is, at the time of this writing, still elusive. Because hemorrhagic deaths, albeit imprecisely defined, most often occur early after injury, and TBI later, time-specific all cause death (i.e., 6-hour and 24-hour death) is currently its best substitute. Thus, RCTs testing hemostatic interventions must focus on early outcomes (e.g., 6–24-hour death).

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Part II

Physiology of Hemostasis

Cell-Mediated Hemostasis

3

Maureane Hoffman

Introduction

In the 1960s two groups proposed a "waterfall" or "cascade" model of coagulation composed of a series of proteolytic steps. Activation of one clotting factor led to the proteolytic activation of the next, finally leading to a burst of thrombin generation [1, 2]. At that time each of the activated factors was thought to have proteolytic activity. We now know that the key components of the coagulation cascade consist of a protease in complex with a non-enzymatic cofactor. While the original models specifically addressed the "intrinsic" pathway, the "coagulation cascade" concept evolved into a Y-shaped scheme, with distinct "intrinsic" and "extrinsic" pathways initiated by factor XII (FXII) and FVIIa/tissue factor (TF) complex, respectively (Fig. 3.1). The pathways converge on a "common" pathway at the level of the FXa/FVa (prothrombinase) complex. The coagulation complexes are generally noted to require phospholipid and calcium for their activity. This scheme was primarily proposed as a model for how the multiple coagulation factors interact to produce a fibrin clot. Of course, hemostasis researchers at the time knew that platelets were important for hemostasis. Platelets were thought primarily to provide lipid

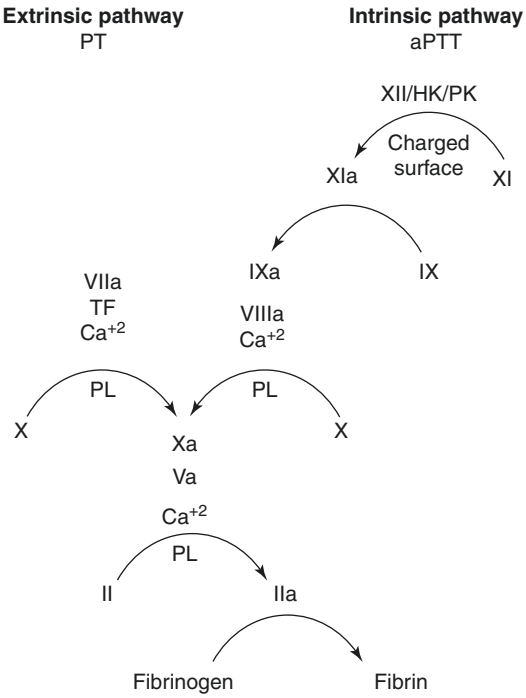


Fig. 3.1 The "extrinsic" and "intrinsic" pathways in the cascade model of coagulation. These two pathways are conceived as each leading to formation of the factor Xa/Va complex, which generates thrombin. *PL* indicates that the reaction requires a phospholipid surface. These pathways are assayed clinically using the prothrombin time (PT) and activated partial thromboplastin time (aPTT), respectively. HK high-molecular-weight kininogen, PK prekallikrein

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membrane surface on which the coagulation complexes were localized. Because platelets are difficult to reproducibly prepare as reagents, they were replaced with phospholipid vesicles in biochemical experiments and subsequently in our common clinical coagulation assays. The cascade model is a good depiction of the sequence of events in the prothrombin time (PT) and activated partial thromboplastic time (aPTT). Furthermore, the concept of a series of proteases acting as a biological amplifier was a breakthrough in understanding hemostasis and a number of other cellular processes, such as complement activation/fixation and apoptosis. However, this protein-centered model failed to account for many facets of hemostasis in vivo.

Many people recognized that the intrinsic and extrinsic systems could not operate in vivo as depicted by this model. For example, it appears in the cascade model as though the "intrinsic" and "extrinsic" pathways are redundant—each leading to the production of activated factor X (FXa). However, patients with a deficiency of FVIII or FIX (hemophilia A or B) have a severe bleeding tendency, even though they have an intact "extrinsic" pathway. Thus, the two pathways are not functionally redundant. In addition, even though deficiencies of each of the factors in the intrinsic pathway can produce long aPTT values, they have dramatically different risks of hemorrhage. Deficiencies of FXII are not associated with significant hemorrhage [3], deficiencies of FXI might or might not be associated with hemorrhage [4], but deficiencies of factors VIII and IX are consistently associated with hemorrhage [5].

Few, if any, medical professionals would assert that contributions from cells do not play a role in hemostasis. However, we sometimes behave as though we think that the results of coagulation screening tests (which only assess the protein components of coagulation) are predictive of the risk of clinical bleeding. The purpose of this chapter is to highlight the roles of cells in localized hemostasis and introduce concepts of how hemostasis can become impaired in vivo.

Cell-Mediated Hemostasis

The key concept underlying the paradigm of "cell-mediated hemostasis" is that cells play active roles in regulating and localizing the coagulation reactions. The complement of receptors and other features of the surfaces of cells are critical to defining the roles of specific cell types in hemostasis. Many cells can participate in hemostasis and thrombosis, but the two critical players are platelets and endothelial cells . Localization of thrombin generation is critical in preventing the spread of clot formation to sites where it is damaging rather than protective. Platelets not only provide the surface on which procoagulant reactions take place, but they also control the rate and localization of thrombin production by adhering specifically to the site of injury. Endothelial cells, on the other hand, have several mechanisms by which they are actively antithrombotic, thus preventing the propagation of clotting from a site of injury throughout the vascular system. A failure of cell-mediated regulation or localization can lead to failures of normal hemostasis, even when the protein components are within normal ranges. This concept seems to be particularly relevant to understanding the mechanisms of bleeding and thrombosis that are induced by trauma. The cell-based experimental model of coagulation [6–8] in combination with existing literature led to the hypothesis [9] that hemostasis occurs as overlapping steps, regulated by cellular components in vivo, as outlined below.

Step 1: Initiation of Coagulation on TF-Bearing Cells

The goal of hemostasis is to produce a platelet and fibrin plug to seal a site of injury or rupture in

the blood vessel wall. This process is initiated when TF-bearing cells are exposed to blood at a site of injury. Tissue factor (TF) is a cofactor that binds to and enhances the activity of its partner protease, FVIIa. TF is structurally related to cytokine receptors, and unrelated to any coagulation factor [10]. This lineage emphasizes the close evolutionary and physiologic links between the coagulation system and components of the host response to injury.

TF is a transmembrane protein [11] that is expressed by epithelial cells, myocardial cells, cerebral cortex, and the connective tissue capsule around most organs [12]. TF is not normally expressed by cells in contact with the blood but is expressed by cells surrounding blood vessels. In the microvasculature myofibroblast-like cells (pericytes) ensheath small vessels and constitutively express high levels of TF. Less well-characterized adventitial cells express high levels of TF around larger arteries and veins. By contrast, many cells express little, if any, TF including skeletal muscle and fibroblasts of the dermis and submucosae. This highly targeted distribution of TF is positioned to rapidly activate coagulation upon injury to vessels or critical organs, and has been described as a "hemostatic envelope."

The TF around vessels is bound to FVII even in the absence of injury [13]. Once bound to TF, zymogen FVII is rapidly activated by autoactivation or by low concentrations of FXa, FIXa, or FVIIa [14]. The FVIIa/TF complexes catalyze activation of small amounts FX and FIX, even in the baseline state. The ongoing generation of small amounts of activated factors means that the coagulation system can be rapidly activated when injury allows blood to enter the extravascular space [15]. Most of the coagulation factors are small enough to leave the vasculature and reside at low levels in the extravascular space, and small amounts of their activation peptides are found in the lymph [16]. However, this baseline activation of coagulation factors is kept separated from other key components of hemostasis by an intact vessel wall. The very large components of the coagulation process are platelets and FVIII bound to multimeric von Willebrand factor (vWF). These components enter the extravascular compartment when an injury disrupts the vessel wall. Escape from the vascular space allows platelets and FVIII-vWF to adhere to collagen and other extravascular matrix components at the site of injury.

The FXa formed on the TF-bearing cell interacts with its cofactor FVa to form prothrombinase complexes and generate a small "priming" amount of thrombin on the TF cells (Fig. 3.2). Experiments using a cell-based model have shown that minute amounts of thrombin are formed in the vicinity of TF-bearing cells exposed to plasma concentrations of procoagulants, even in the absence of platelets. The small amounts of FVa required for prothrombinase assembly on TF-bearing cells can be activated by FXa [17] or by non-coagulation proteases produced by the cells [18]. However, thrombin generation during initiation of coagulation in vivo is predominantly supported by the release of FV(a) from platelets that adhere to the site of injury [19]. Platelets release a distinct form of FV that has been taken up from plasma and processed to make it active as a procoagulant and resistant to inactivation by protein C [20]. Thus, a collaboration between the TF-bearing cells and "first responder" platelets leads to the production of the small amount of thrombin that is critical to the amplification phase.

The factors Xa and IXa formed on TF-bearing cells have very distinct and separate functions in blood coagulation [8]. The activity of FXa formed by the FVIIa/TF complex is restricted to the TF-bearing cell because FXa that dissociates from the cell surface is rapidly inhibited by tissue factor pathway inhibitor (TFPI) or antithrombin (AT) in the fluid phase (Fig. 3.3). In contrast, FIXa can diffuse through the fluid phase because it is not inhibited by TFPI and is inhibited much more slowly by AT than FXa. FIXa that reaches the surface of nearby activated platelets can bind to a specific receptor [21], and participate in platelet surface thrombin generation during the propagation phase.

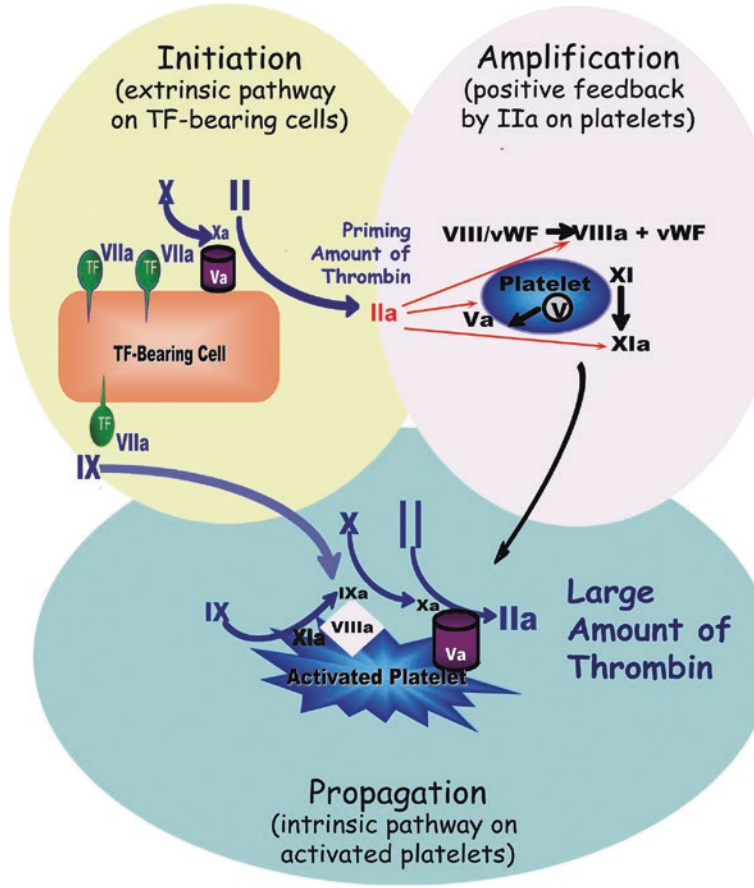


Fig. 3.2 Steps in a cell-based model of coagulation. Initiation occurs on the TF-bearing cell as activated FX combines with its cofactor, FVa, to activate small amounts of thrombin. The priming amount of thrombin generated on the TF-bearing cell amplifies the procoagulant response by activating cofactors, factor XI and platelets. The large burst of thrombin required for effective hemostasis is formed on the platelet surface during the propagation phase. (Modified from Ref. [52])

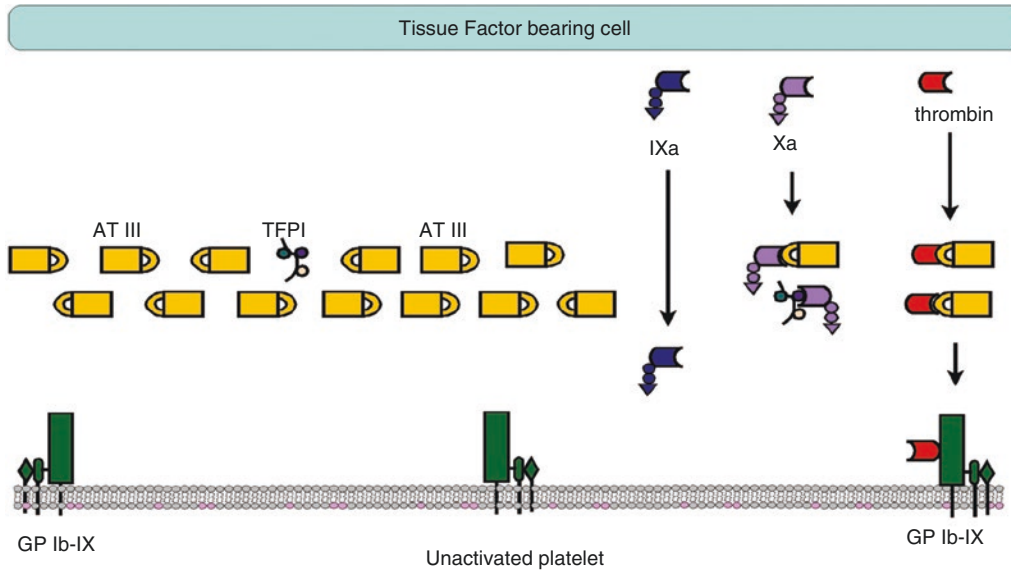


Fig. 3.3 The coagulation inhibitors, antithrombin (AT) and tissue factor pathway inhibitor (TFPI), provide a major barrier to the movement of activated coagulation proteases between cell surfaces. Thus, they tend to localize procoagulant activity to the surface on which it was formed. However, FIXa has a greater ability to diffuse between cell surfaces, since it is not inhibited by TFPI and is much more slowly inhibited by AT than are thrombin (IIa) or FXa

Step 2: Amplification of the Procoagulant Signal

Binding of platelets to the site of injury leads to partial platelet activation. This is probably initiated by binding to collagen in the extracellular matrix. As platelets are activated, they release adenosine diphosphate (ADP) from their dense granules, which potentiates activation of additional platelets. Thrombin is a potent platelet activator both through glycoprotein (GP) Ib and the protease activated receptor (PAR)-1 [22–24]. PAR-4 also contributes to platelet activation by thrombin [23]. Simultaneous engagement of platelet receptors by both collagen and thrombin leads to the activation of platelets to a highly procoagulant state [25]. These platelets have been referred to as COAT (collagen and thrombin stimulated) platelets [26]. The greatest procoagulant activity is likely generated on the first platelets to arrive at the site of injury. Those are the platelets that will have both bound to collagen and also been exposed to thrombin. Once the exposed collagen matrix is covered by a platelet/fibrin layer, additional platelets that accumulate would not be activated to the "COAT" state—thus damping down the coagulation process as the area of the wound is covered by a hemostatic clot.

The activated platelets initially provide a surface for the feedback activation of coagulation factors V, VIII, and XI by thrombin and amplification of the initial procoagulant stimulus. GPIb not only acts as a receptor mediating thrombin activation of platelets, but it also serves as a scaffold that binds FVIII-vWF and FXI [27] and facilitates their activation by thrombin. Activation of FXI by thrombin is not very efficient. However, it has recently been found that polyphosphate released from platelet dense granules enhances the rate of FXI activation by thrombin about 3000-fold [28]. Thus, the activation of FXI by thrombin in association with platelets explains why FXII is not essential for hemostasis [29]. Platelet polyphosphate also enhances the rate of FV activation [30]. Although the small amount of thrombin generated during the initiation phase may not be sufficient to clot fibrinogen, it drives positive feedback circuits that set the stage for a subsequent large burst of thrombin generation by: (1) fully activating platelets; (2) activating FV; (3) activating FVIII and dissociating it from vWF; and (4) activating FXI .

Step 3: Propagation of Thrombin Generation on the Platelet Surface

Platelets play a major role in localizing clotting reactions, since they adhere to and aggregate at the sites of injury where TF is also exposed. They provide the primary surface for generation of the burst of thrombin needed for effective hemostasis during the propagation phase of coagulation. Once platelets are activated, the cofactors FVa and FVIIIa are rapidly localized on the platelet surface [7]. As noted above, the FIXa formed by the FVIIa/TF complex can diffuse through the fluid phase and also bind to the surface of activated platelets. FXI that was activated by the "priming" amount of thrombin can activate more FIX to IXa, supplementing the initial activation of FIX provided by FVIIa/TF. Once the platelet "tenase" (FIXa/FVIIIa) complex is assembled, FX from the plasma is activated to FXa on the platelet surface. FXa then associates with FVa to support a burst of thrombin generation of sufficient magnitude to produce a stable fibrin clot. The fibrin clot enmeshes and stabilizes the initial platelet plug for the time required for tissue repair to take place.

The large amount of thrombin generated on platelet surfaces is responsible for stabilizing the hemostatic clot in more ways than just initiating fibrin polymerization. In fact, most of the thrombin generated during the hemostatic process is produced after the initial fibrin clot is formed [31]. The burst of thrombin also stabilizes the clot by: (1) activating FXIII [32], which then crosslinks fibrin monomers; (2) activating thrombin-activatable fibrinolysis inhibitor (TAFI) [33], which reduces plasmin activation on the fibrin clot; (3) cleaving the platelet protease activated receptor (PAR)-4 receptor [34], which requires a higher concentration of thrombin than does PAR-1 [35]; and (4) being incorporated into the structure of the clot, where it can rapidly reactivate the coagulant response if the initial clot is disrupted.

Even though each phase of the cell-based model has been described as a discrete event, they should be viewed as an overlapping continuum. For example, thrombin produced on the platelet surface early in the propagation phase may initially cleave substrates on the platelet surface and continue to amplify the procoagulant response, in addition to leaving the platelet and promoting fibrin assembly. The cell-based model of coagulation shows us that the "extrinsic" and "intrinsic" pathways are not redundant. Let us consider the "extrinsic" or pathway to consist of the FVIIa/TF complex working with the FXa/Va complex and the "intrinsic" pathway to consist of FXIa working with the complexes of factors VIIIa/IXa and factors Xa/Va. The "extrinsic" pathway operates on the TF-bearing cell to initiate and amplify coagulation. By contrast, the "intrinsic" pathway operates on the activated platelet surface to produce the burst of thrombin that leads to formation and stabilization of the fibrin clot.

What Controls Coagulation and Stops It Once It Gets Started?

Localizing production of thrombin to specific cell surfaces is critical to preventing uncontrolled coagulation, leading to thrombosis or disseminated intravascular coagulation (DIC). Receptors on cells play an important role in localization. In addition, several plasma proteins play critical roles in limiting the coagulation process and restricting large-scale thrombin generation to the surface of activated platelets. These include antithrombin (AT, previously called antithrombin III), tissue factor pathway inhibitor (TFPI), and the protein C/S system. AT and TFPI are important in localizing the activity of coagulation complexes to specific cell surfaces and preventing coagulation activation in the circulation and on endothelial surfaces. Protein C and protein S are important in inactivating procoagulant complexes on endothelial surfaces.

AT is an inhibitor, not only of thrombin, but also of all of the coagulation proteases. Its effectiveness as an inhibitor is enhanced in the presence of heparin-like molecules. Proteases on cell surfaces are less susceptible to inhibition by AT. Thus, the presence of AT in the fluid phase tends to limit the activity of the coagulation factors to the surface on which they were activated. However, AT can exert its effects on specific cell surfaces. The presence of heparan sulfates is thought to localize AT and enhance its activity on endothelial cells, thus contributing to the actively antithrombotic nature of healthy endothelium.

TFPI is a protease inhibitor that proves to have a quite complex biology [36]. It is a direct inhibitor of FXa and, when in complex with FXa, can also inhibit the FVIIa/TF complex. TFPI is a very effective inhibitor of FXa in solution and thus tends to limit FXa activity to the surface on which it was formed. Once FVIIa/TF has activated some FXa, TFPI/Xa is thought to act as a negative feedback regulator and inhibit further FVIIa/TF activity. This would tend to limit the duration of the procoagulant stimulus after TF is exposed to blood. TFPI is also localized to endothelial cells, in addition to circulating in the plasma. One form of TFPI (TFPI-alpha) is localized to endothelial cells by binding to heparan sulfates. TFPI-alpha can effectively inhibit the FXa/FVa (prothrombinase) complex and limit initiation of large-scale thrombin generation at sites where the procoagulant stimulus is weak [37]. A second form (TFPI-beta) is covalently attached to the endothelial surface by glycosylphosphatidylinositol (GPI) linkage. Thus, TFPI isoforms make important contributions to the actively anticoagulant nature of the endothelium, as well as limiting the procoagulant effects of FVIIa/TF.

The protein C/S system is also very important in preventing activation of coagulation on healthy endothelium. Thrombin is appropriately produced at a site of vascular injury. However, the endothelium has mechanisms to prevent generation of thrombin on its surfaces. Most endothelial cells express the endothelialprotein C receptor (EPCR) and thrombomodulin (TM)—both transmembrane proteins. As illustrated in Fig. 3.4, thrombin which diffuses away from a site of injury, and onto healthy endothelium, binds to TM. Protein C from the plasma binds to EPCR where it is activated by the thrombin/TM

A L G r a w a n y

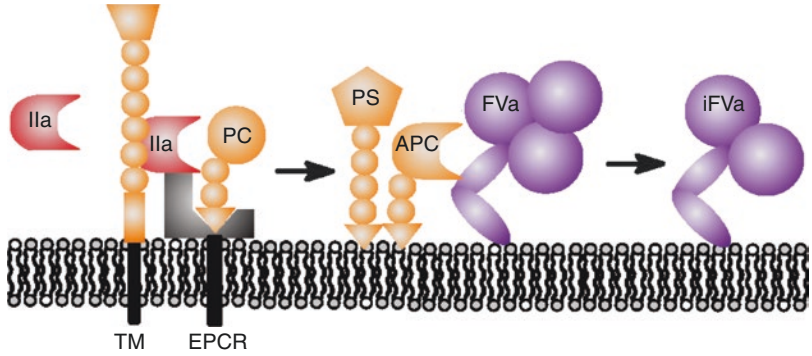


Fig. 3.4 The protein C system has an antithrombotic effect by preventing activation of thrombin on endothelial surfaces. Thrombin (IIa) that reaches the surface of healthy endothelium binds to thrombomodulin (TM) which allows it to activate protein C (PC) that has bound to the endothelial protein C receptor (EPCR). activated protein C (APC) in concert with its cofactor, protein S (PS), cleaves and inactivates FVa and FVIIIa (not shown). This effectively prevents assembly of procoagulant complexes on normal endothelial surfaces

complex. Activated protein C (APC), in concert with its cofactor protein S, cleaves and inactivates FVa and FVIIIa (not shown in Fig. 3.4), thus preventing additional generation of activated factors on normal endothelial surfaces. Components of the protein C system can also play a role in derangements of the coagulation system. For example, levels of both EPCR and TM on the endothelial surface are reduced as a consequence of inflammation, thus predisposing to thrombosis [38, 39]. By contrast, systemic activation of protein C appears to play a role in the early coagulopathy of trauma, which will be discussed in detail elsewhere in this volume [40].

Step 2: Amplification of the Procoagulant Signal by Thrombin Generated on the TF (Fig. 3.4)

The protein C system has an antithrombotic effect by preventing activation of thrombin on endothelial surfaces. Thrombin (IIa) that reaches the surface of healthy endothelium binds to thrombomodulin (TM) which allows it to activate Protein C (PC) that has bound to the endothelial protein C receptor (EPCR). Activated protein C (APC) in concert with its cofactor, protein S (PS), cleaves and inactivates FVa and FVIIIa (not shown). This effectively prevents assembly of procoagulant complexes on normal endothelial surfaces.

Many have considered the protein C system to be the mechanism by which hemostatic coagulation reactions are terminated. Certainly extension of coagulation through the vascular tree is limited by the action of APC, as well as by AT and TFPI. However, it seems more likely to us that cleavage of cofactors by APC is not the mechanism for ending thrombin generation on platelet surfaces. This is because there is not a good mechanism to localize APC to platelets as there is to endothelial cells, and because FVa on platelet surfaces is resistant to inactivation by APC [20, 41]. It seems more likely that hemostatic coagulation is terminated, at least in part, by "paving over" the highly procoagulant "COATed" platelets that are bound to collagen, with layers of less highly procoagulant platelets. Therefore, we think of the protein C system under normal conditions as being a relatively endothelial-specific antithrombotic mechanism, rather than a general anticoagulant.

Fibrinolysis

Fibrinolysis is essential for removal of clots during the process of wound healing as well as for removing intravascular clots that might otherwise be manifested as thrombosis. Intravascular deposition of fibrin is also associated with the develop-

ment of atherosclerosis. Therefore, an effective fibrinolytic system tends to protect against the chronic process of atherosclerotic vascular disease as well as the acute process of thrombosis. Conversely, defects of fibrinolysis increase the risk of atherothrombotic disease. For example, elevated levels of plasminogen activator inhibitor-1, an inhibitor of fibrinolysis, are associated with an increased risk of atherosclerosis and thrombosis [42] as are decreased levels of plasminogen [43]. Thus, effectiveness of hemostasis in vivo depends not only on the procoagulant reactions, but also on the fibrinolytic process. In the case of trauma, the coagulation reactions are in a race with the fibrinolytic process to form a clot stable enough to persist until healing can occur. In some cases this situation can be detected as a systemic hyperfibrinolytic state on whole blood testing, such as with the thromboelastogram. However, this situation is more often local, with plasmin activation occurring primarily at the site of injury and not necessarily being detectible in the blood. For further reading on the mechanism of plasmin/anti-plasmin system, please refer to Chap. 3 and pathologic fibrinolysis from trauma in Chap. 9.

Hemostasis Is Not the Same in All Tissues

The above sections describe components of the hemostatic response as though it were the same in all tissues. However, alterations in the hemostatic system almost invariably produce localized lesions, suggesting that hemostasis is regulated in a tissue-specific manner. The tissue-specific properties of hemostasis can be due to differences in vascular beds, tissue structure, and the levels of procoagulant, anti-thrombotic, and fibrinolytic components in a particular tissue [44–46].

What Does All This Mean for Clinical Laboratory Testing?

It should be clear from the preceding discussion that commonly used clinical coagulation tests do not really reflect the complexity of hemostasis in vivo. That does not mean that the PT and aPTT are useless. We just need to understand their limitations. These "screening" coagulation tests are abnormal when there is a deficiency of one or more of the soluble coagulation factors. They do not tell us what the risk of clinical bleeding will be. As noted before, two patients with identical aPTT values can have drastically different risks of hemorrhage (XII vs XI vs IX vs VIII). All of our common coagulation tests including the PT, aPTT, thrombin clotting time, fibrinogen levels, and coagulation factor levels tell us something about the plasma level of soluble factors required for hemostasis. Their clinical implications must be evaluated by the physician ordering these tests. Thus, just because the PT and aPTT are within the normal range, it does not follow that the patient is at no risk for bleeding. Conversely, a mild elevation in these clotting times does not mean that the patient is at risk for bleeding after an invasive procedure.

Several whole blood ("global") coagulation tests are jockeying for position as a means of evaluating overall hemostatic status in selected clinical settings. While whole blood tests have the advantage that they may reflect the contributions of platelets and other blood cells to the hemostatic process, they still do not reflect the contributions of the tissue cells and local tissue conditions. Furthermore, the reagents used to initiate the coagulation reactions in these may not be very physiologically relevant. Thus any laboratory test requires skilled interpretation and clinical correlation in evaluating the true risk of bleeding. For further reading on these tests, please refer to Chaps. 17 and 18.

What Causes Bleeding in Previously Normal Patients?

Many patients that experience significant hemorrhage do not have an underlying bleeding tendency that can be identified prior to a bleeding episode. However, a bleeding tendency (coagulopathy) may develop as a result of the episode of hemorrhage. It is important to recognize that mechanical control of bleeding is the first priority in trauma. With ongoing bleeding due to surgical or acciden-

tal trauma, the risk of developing systemic coagulopathy increases as resuscitation and transfusion alone fail to address the underlying cause of the hemorrhage. The hallmark of coagulopathy is microvascular bleeding. In spite of being characterized by bleeding from very small vessels, microvascular bleeding can lead to massive blood loss, which is difficult to control mechanically. Drivers of coagulopathic bleeding include consumption of coagulation factors and platelets, dysregulation of endothelial protective mechanisms including the protein C/S system, excessive fibrinolysis, hypothermia, and acidosis. While many contributors to trauma-associated coagulopathy have been identified, the relative role of the various mechanisms differs between patients. It appears that a variety of mechanisms can ultimately result in a similar systemic coagulopathy.

Consumption of Coagulation Components

We normally think of disseminated intravascular coagulation (DIC) when we talk of consumption. In DIC the normal localization of coagulation fails, and coagulation factors are activated throughout the circulation. This leads to production of microthrombi and also consumption of platelets, coagulation factors, and coagulation inhibitors. However, clotting factors and platelets can also be consumed during appropriate physiological attempts at hemostasis. In this case it is appropriate to replace the depleted factors with transfusion therapy.

DIC can be much more complicated to manage [47]. The mainstay of treatment is to treat the underlying disorder, such as sepsis. DIC can also be triggered by tissue damage due to trauma. In early or mild/compensated DIC administration of low dose heparin may be considered to control the procoagulant response to inflammation, infection, or malignancy. However, in more severe or advanced DIC replacement therapy may be necessary to treat the bleeding tendency associated with the depletion of coagulation factors and platelets. Please refer to Chap. 13 for further reading on DIC management from trauma.

Dilution of Coagulation Components

In the face of significant hemorrhage, replacement of blood loss with non-blood solutions can result in dilution of the coagulation factors, platelets, and inhibitors. Thrombin generating ability is well preserved even when the coagulation factors and inhibitors are diluted by as much as 50%. For continued reading on techniques to reduce dilution coagulopathy, refer to the massive transfusion in Chap. 23.

Hypothermia

Many patients become hypothermic during medical illness or following surgical or accidental trauma [48]. Hypothermia can directly interfere with the hemostatic process by slowing the activity of coagulation enzymes. Platelet adhesion and aggregation is also impaired, even in mild hypothermia [49]. Thus, hypothermic patients will have some degree of impairment in platelet function and coagulation enzyme activity even without other causes of coagulopathy.

Acidosis

Acidosis can have an even more profound effect on procoagulant function than hypothermia, though the two metabolic abnormalities often coexist. A drop in the pH from 7.4 to 7.2 reduces the activity of each of the coagulation proteases by more than half [50]. Thus, acidosis should be considered as a possible contributor to coagulopathic bleeding in medical and surgical patients.

What Happens After the Bleeding Stops?

Once hemostasis is completed, the process of wound healing begins. The hemostatic plug must be stable enough to maintain hemostasis until healing is well under way, yet be removed so as not to interfere with tissue repair. At this point

fibrinolysis is essential, through the action of plasmin in concert with leukocyte proteases. The neutrophils that initially accumulate at a site of injury are replaced over the course of a few days with macrophages that engulf and degrade cellular debris and components of the fibrin clot. The macrophages secrete cytokines and growth factors that facilitate the migration of fibroblasts and endothelial cells into the wound site. In the case of a skin wound, the dermis is replaced by highly cellular and vascular granulation tissue, while the surface epithelium proliferates and migrates from the margins to cover the surface of the wound. Many of the activities involved in wound healing are influenced by thrombin. Thrombin plays a major role in platelet activation and degranulation. Several key cytokines modulating wound healing are released from activated platelets, including transforming growth factor beta (TGFβ1), and platelet-derived growth factor (PDGF). Of course, the amount and rate of thrombin generated during hemostasis influence the initial structure of the fibrin clot—the framework on which cell migration takes place. In addition, thrombin has chemotactic and mitogenic activities for macrophages, fibroblasts, smooth muscle cells, and endothelial cells. Impaired hemostasis leads to a delay in wound healing [51]. Thus, generation of the "right" amount of thrombin during the coagulation process is not only essential for effective hemostasis but sets the stage for effective wound healing.

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Thrombin-Antithrombin System

4

Toshiaki Iba

Introduction

Antithrombin (AT) is the most abundant and most important physiological anticoagulant in the body [1]. AT deficiency in animal results in embryonic death, and mutations that reduce the AT levels result in severe thrombotic conditions. For example, AT-deficient fish exhibited consumptive coagulopathy or disseminated intravascular coagulation (DIC) following injury [2]. It is generally accepted that thrombin is produced as a result of activation of the innate immune system after injury, and natural anticoagulants play a role in preventing accidental clot formation. Since ancient times massive blood loss from trauma has been the most frequent life-threatening event encountered by humans. As a result, a heavy-duty coagulation system has evolved to promote hemostasis in the setting of injury. In the face of this natural thrombogenic tendency, impairment of anticoagulant mechanisms can easily induce problematic events in critical situations, including trauma [3]. As a result, the dysregulated overactivation in coagulation tends to occur under such circumstances. The disorder is represented by massive microvascular thrombi that contribute to decreased oxygen delivery and subsequent organ dysfunction. Accordingly, anticoagulant therapy is expected to play some role in alleviating this dangerous coagulation disorder [4]. Large-scale randomized controlled trials (RCTs) to examine the effects of anticoagulant therapies were conducted in the early 2000s, predominantly focused on sepsis [5–7], but none of the anticoagulant agents examined demonstrated a clear beneficial effect. As a result, no anticoagulant is currently approved for the clinical use in the treatment of coagulation disorder or DIC. However, some clinical trials have suggested that sepsis-associated DIC, but not sepsis itself, could benefit from anticoagulant therapies. Some subgroup analyses performed among the subjects with sepsis-associated DIC in the aforementioned clinical trials have revealed trends toward favorable effects of activated protein C and AT on the mortality [8, 9]. Recently, a small, but well-designed RCT succeeded in demonstrating the efficacy of a physiological dose of AT for obtaining DIC resolution in septic patients with DIC [10]. In addition, recombinant thrombomodulin has also shown a trend toward beneficial effects on the mortality in sepsis patients with coagulation disorder [11]. Analyses using a nationwide administrative database of patients with DIC in Japan revealed positive effects of AT supplementation on the mortality [12, 13]. However, there is still no concrete evidence of the efficacy of AT in patients with trauma-induced coagulopathy (TIC). In the following part of this

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chapter, the structure, unique characteristics of this naturally derived anticoagulant, and the theoretical background for the use of AT in systemic coagulation disorders are introduced.

Structure and Function of Antithrombin

AT, a 432-amino acid glycoprotein, with a molecular weight of approximately 58 kDa, is found in the circulating blood at a concentration range of 125–150 μg/mL. AT is mainly produced by the liver and its reported half-life is 2.8 days under physiological conditions, although it is known to be significantly shortened under various stresses [14, 15]. AT acts as a serine protease inhibitor and inactivates the enzymatic activity of serine protease coagulation factors, including factors VIIa, IXa, Xa, XIa, and IIa, by forming a 1:1 complex [16] (Fig. 4.1). In the case of thrombin (factor IIa), AT binds to thrombin and leads to the formation of a thrombin-antithrombin complex (TAT), which results in the inactivation of the enzymatic activity of thrombin and its elimination from the circulation (Fig. 4.2). Approximately 10% of the total AT is bound to the heparan sulfate of glycosaminoglycans on the vascular endothelial surface, 40% circulates in the plasma, and 50% is distributed in the extravascular space. The anticoagulant activity of AT is known to increase by a thousand-fold or more after it is bound to heparin and heparinoids. This is due to a conformational change with extrusion of the reactive center loop to yield a more

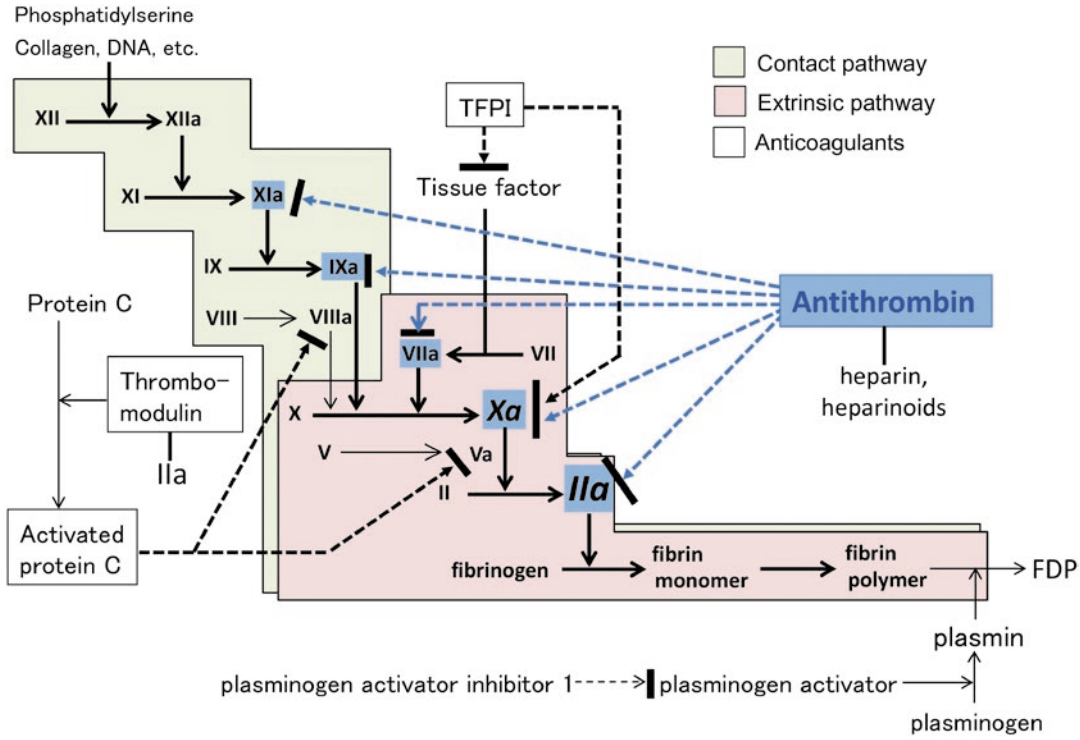


Fig. 4.1 Anti-coagulant activities of antithrombin (AT). Systemic activation of coagulation is commonly seen in cases of major trauma. Procoagulant substances released from damaged tissues, as a result of tissue hypoxia and vascular injury, are involved in this physiologic reaction. There are three major anticoagulant systems, i.e., the AT-heparin and heparinoid system, the thrombomodulin-protein C system, and the tissue factor pathway inhibitor (TFPI) system. Among them, the AT-heparin and heparinoid system is the most prominent and plays vital roles. AT acts as a serine protease inhibitor and inactivates multiple serine protease coagulation factors, such as factors VIIa, IXa, Xa, XIa, and IIa

exposed orientation to bind coagulation factors [15]. AT has four potential N-glycosylation sites on asparagine (Asn) 96, 135, 155, and 192 (Fig. 4.3). There are two glycoforms of AT, α- and β-AT; while all of the four N-glycosylation sites are occupied by covalently attached oligosaccharide side-chains in the predominant form of AT, namely, α-AT (>90%), only three glycosylation sites are occupied in β-AT (<10%), and Asn 135 is not glycosylated in this form [17,

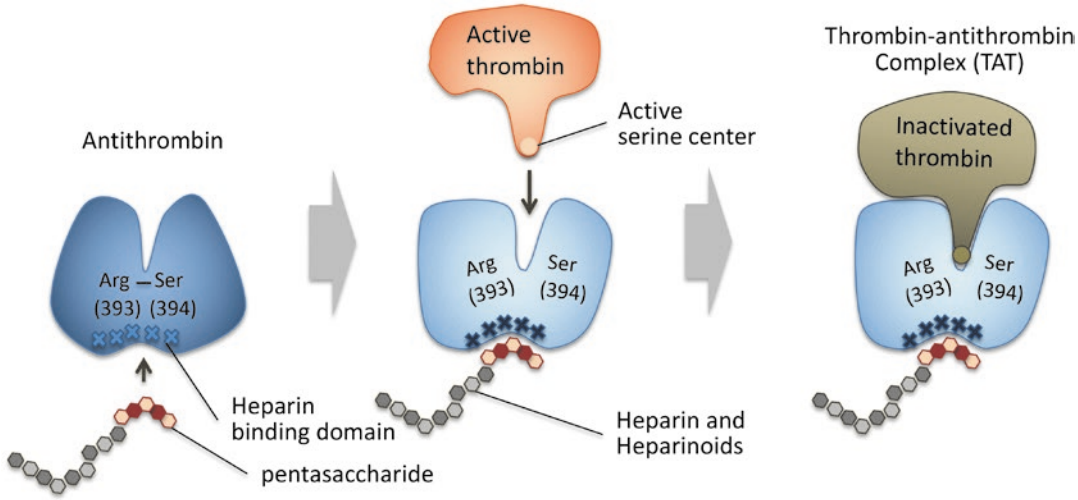


Fig. 4.2 The binding of antithrombin (AT) to thrombin. AT contains a heparin-binding domain, and the anticoagulant activity of AT is increased by more than a 1000-fold when it is bound to the pentasaccharide structure of heparin or heparinoids. This binding of heparin to AT induces the conformational change of AT that results in an increase in its affinity to thrombin. AT inhibits thrombin in a 1:1 fashion and forms a thrombin-antithrombin complex (TAT), thereby inactivating the enzymatic activity of thrombin. The formation of the TAT complex involves interaction between the protease (thrombin) and a specific reactive peptide bond (between arginine (Arg) 393 and serine (Ser) 394) within AT

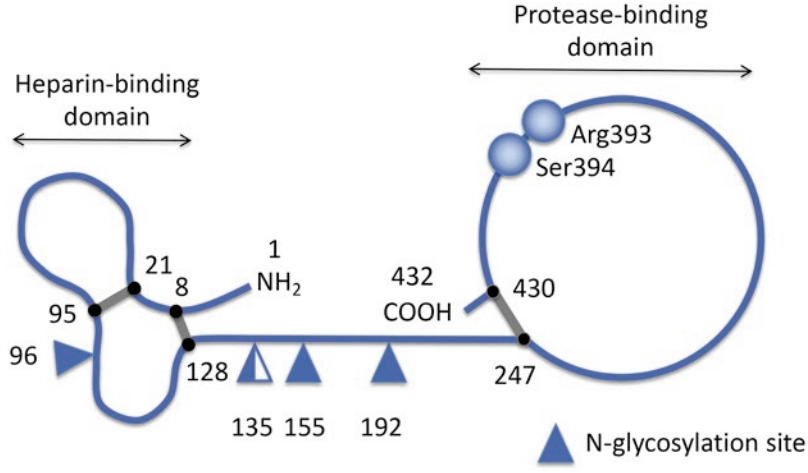


Fig. 4.3 The structure of antithrombin (AT). AT is a glycoprotein that is composed of 432 amino acids, with a molecular weight of approximately 58 kDa. AT has four N-glycosylation sites on asparagine (Asn) 96, 135, 155, and 192. There are two glycoforms, α- and β-AT. All of the four N-glycosylation sites of α-AT are occupied by covalently attached oligosaccharide side-chains, while only three N-glycosylation sites of β-AT are occupied, and Asn 135 is not glycosylated. As compared to the predominantly occurring form of α-AT (>90%), β-AT has a higher affinity to heparin and heparinoids, and the binding induces a conformational change of the protease-binding domain of AT

18]. As a result, the molecular weight of β-AT is slightly lower than that of α-AT. Although β-AT occurs at lower levels, it shows a higher affinity to the pentasaccharide component of heparin and heparan sulfate of the glycocalyx, and a stronger direct inhibitory activity on thrombin and factor Xa. Therefore, β-AT is considered to be the more important form of AT for preventing thrombus formation in the vascular system [17, 18].

Like other natural proteins, AT is also a pluripotent substance, that not only acts as an inhibitor of multiple coagulation factors but also exerts anti-inflammatory actions, and the latter is expressed through both anticoagulation-dependent and anticoagulation-independent mechanisms [1, 19]. The anti-inflammatory activity of AT can be explained partly by its neutralization of thrombin. Thrombin has been implicated in the inflammatory cascade [20]; first, thrombin can induce leukocyte rolling and adhesion to the vascular endothelium [21] by increasing the expression of selectin [22, 23] and intercellular adhesion molecule-1 [24], thereby promoting leukocyte recruitment. In addition, AT also exerts anti-inflammatory activity via through coagulation-independent mechanisms. Thrombin can elicit an inflammatory reaction through binding to its receptor, protease-activated receptor (PAR)-1 [25]. PAR-1 plays a pivotal role in the activation of inflammation by inducing the production of proinflammatory cytokines and chemokines by the leukocytes and endothelium [26, 27] (Fig. 4.4). AT mitigates the inflammatory reactions, especially when it binds to its specific

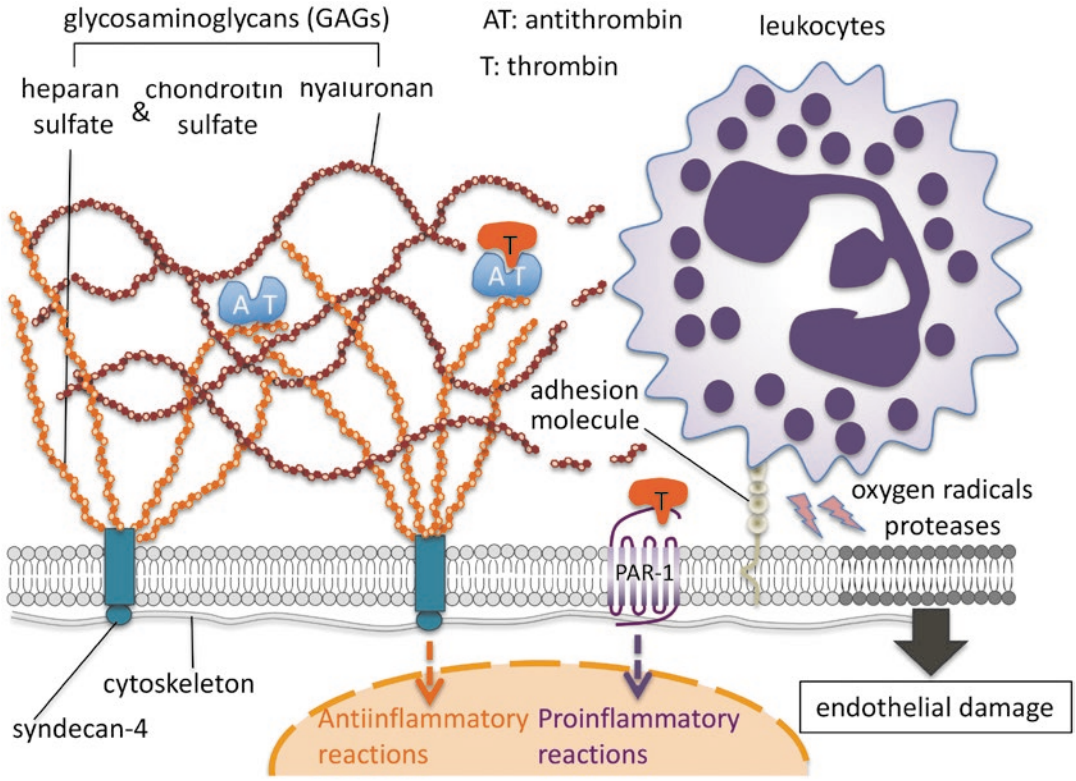


Fig. 4.4 Multifactorial functions of antithrombin (AT) on the endothelial cell. The interactions between antithrombin (AT) and the endothelium are shown. The affinity of AT to thrombin and its enzymatic inhibition is increased by its binding to heparan sulfate, a major component of the glycosaminoglycans (GAGs). Heparan sulfate is a side chain of syndecan-4, a transmembrane proteoglycan on the cell surface, and information on the binding of AT to heparan sulfate elicits an anti-inflammatory reaction on the endothelium. On the contrary, the binding of thrombin to its receptor, protease-activated receptor (PAR)-1, on the endothelium elicits a proinflammatory reaction

receptor, heparan sulfate, a type of heparin-like glycosaminoglycan (GAG) [28]. Heparan sulfate is a side chain of syndecan-4, a transmembrane proteoglycan that transmits and induces endothelial reactions, including the production of vasoactive substances such as prostacyclin. Prostacyclin exerts anti-inflammatory activity by blocking neutrophil tethering on the endothelium, limiting vasoconstriction [29], and downregulating the production of pro-inflammatory cytokines [30].

Usefulness of Antithrombin as a Biomarker

The plasma levels of AT antigen and AT activity can be measured either by ELISA or by a functional assay. The prognostic value of AT activity for predicting the morbidity and mortality has been repeatedly reported in cases of sepsis-associated DIC. Indeed, the usefulness of AT measurement is superior to those of the results of global coagulation tests such as prothrombin time, levels of fibrin/fibrinogen degradation products, D-dimer levels, and platelet counts [31]. The measurement of the baseline value of AT is recommended for selecting appropriate candidates for anticoagulant therapy, and monitoring of the sequential changes in AT levels during supplementation therapy is also reportedly helpful to estimate the prognosis [31].

Similar to the case in sepsis, low AT levels are also associated with the risk of coagulopathy and thromboembolic complications in trauma patients [32, 33]. The severity of the injury, increase in thrombin generation, amount of blood loss, and tissue hypoperfusion are considered to be the major contributors to the decrease of the AT levels in trauma patients [34]. Extremely low levels of AT immediately after arrival to the emergency room are seen in very severe cases of trauma and coagulopathy, with the reduced levels of AT sustained for several days after admission [35–37]. In a recent study, Ohshiro et al. [38] reported that trauma patients with DIC showed consumptive coagulopathy, lower AT levels, and higher levels of fibrin/fibrinogen degradation products and D-dimers as compared to trauma patients without DIC, and all of these patients with DIC had complicating organ dysfunction and required higher volumes of blood transfusion and are therefore associated with substantially worse outcomes as compared to the non-DIC trauma patients. They also emphasized that the coagulation control afforded by AT is insufficient to anchor thrombin at the injured site after the accident in patients with severe trauma and coagulopathy. Similar observations were reported in patients with brain injury [39]. Especially in patients with severe head injury, the endothelial activation that is associated with the expression of intravascular tissue factor followed by thrombin formation is well known, and in addition, induction of hyperfibrinolysis by the release of tissue-type plasminogen activator from the injured brain tissues is reported [40]. Thus, the coagulation disorder with a decrease in AT activity is more pronounced in cases with brain trauma.

Apart from the plasma AT antigen level or AT activity described above, TAT levels can also be measured in the plasma. TAT is formed as a result of inactivation of thrombin and is recognized as a sensitive marker for activation of coagulation. Serial measurements in trauma patients have revealed marked elevation of the TAT levels on the first day of the injury, corresponding to the decreased AT activity and expression of tissue factor by the injury [41, 42]. TAT levels usually decrease by days 2–4 after the trauma, if the injuries are managed appropriately. It is noteworthy that the TAT levels were influenced by both thrombin and AT levels, and therefore, decreased TAT levels do not always indicate a decrease of thrombin generation but also reflect the decreased AT levels.

Antithrombin Therapy in Animal Models

In animal models of trauma, DIC with the fibrinolysis phenotype followed by the thrombosis phenotype can be reproduced. Such models show decreased platelet counts, prolonged prothrombin times, elevated levels of fibrin/fibrinogen degradation products, and decreased AT levels

A L G r a w a n y

[43]. It was found in a pig model of coagulopathy with blunt liver injury that the decreased AT level was not sufficient to quench the increased thrombin generation [44]. In another model, the acute-phase activation of fibrinolysis represented by elevation in the levels of tissue-type plasminogen activator was reproduced, and a reduction in the level of α2-antiplasmin is reported [45]. Thus, it is strongly suggested that tissue factor released from damaged tissues into the circulation plays a pivotal role in these models. Consequently, activation of the extrinsic coagulation pathway is considered to be the predominant mechanism underlying the pathogenesis of coagulopathy following trauma, and thrombin is the vital mediator [46]. This type of coagulation disorder easily leads to consumptive coagulopathy, together with the decrease in the levels of anticoagulants such as AT and protein C. At the same time, the antifibrinolytic factor α2 antiplasmin is commonly depleted and hyperfibrinolysis occurs [47, 48]. Therefore, the usefulness of supplementation of anticoagulants can be expected; however, animal experiments performed to examine the effects of AT on TIC are unexpectedly scarce. The effects of anticoagulants have mainly been examined in models of sepsis, burns, and venom poisoning [49]. While the antithrombotic effects of AT and heparin were examined in a rabbit model of vascular injury, and only animals given both AT and heparin showed inhibition of thrombin generation and fibrin deposition on the aortic surface [50]. This study revealed only the antithrombotic effect and not the pure anticoagulant effects of AT, and direct evidence to show the efficacy of AT on TIC is still missing.

The effects of AT should be studied more intensively because not only beneficial effects on TIC but also the anti-inflammatory effects of AT can be expected. Acute lung injury was induced in a rat model of crush injury, and treatment with AT significantly mitigated the vascular damage. In the AT-treated animals, all of the accumulation of inflammatory cells, expression of von Willebrand factor, and expression of vascular endothelial cell adhesion molecule 1 were attenuated, and these anti-inflammatory effects potentially lead to a decrease in the mortality [51]. In addition to the above, anti-inflammatory effects of AT exerted through stimulation of prostaglandin I_2 was also reported in a rat model of spinal cord injury [52].

AT is also used to prevent thrombotic events in patients treated with prothrombin complex concentrates. Recently, prothrombin complex concentrates have been used increasingly as a part of the management of bleeding in trauma patients. There are potential risks of thromboembolic complications in these cases. The antithrombotic property of AT is plausible, and consequently, the effect of AT in the prevention of thrombosis was reported in a porcine model of polytrauma treated with prothrombin complex concentrate [53].

Antithrombin Therapy in Trauma Patients

Since increased levels of circulating thrombin are the major cause of activated coagulation in trauma patients, the use of AT is seemingly a correct approach. Some studies have reported that decreased AT levels failed to control the hemostasis at local wound sites and enhanced systemic thrombin generation in patients with trauma [47, 48]. In addition, it has also been demonstrated in trauma patients with DIC that the decrease in AT activity is an independent determinant of elevated levels of soluble fibrin, a marker of thrombin generation [37]. Owings et al. [54] reported that procoagulant markers such as the levels of prothrombin fragment $_{1.2}$, TAT, and D-dimer were all significantly elevated in patients with severe injuries, along with a decrease of the AT level in patients with severe injury; however, the levels of AT and protein C were not different in the total population in this study. They also reported significantly reduced AT levels in patients with coagulopathy and acute respiratory distress syndrome and concluded that the reduced availability of AT and protein C is the major threats in trauma patients. Despite the important roles of AT in trauma, no RCTs have been performed yet to examine the effects of AT in TIC, although the effects of AT supplementation in patients in a critical condition, including trauma patients, have been examined in some trials [55–57]. The hypothesis in these trials was that normalization

of AT activity by AT supplementation reduces the mortality in trauma patients. However, none of the trials showed any improvement in the outcomes. Nevertheless, it has been reported that AT supplementation permitted better maintenance of the clotting system in one trial [55]. Another trial suggested that AT therapy may reduce the incidence of renal impairment [56], and another study reported the potential efficacy of high-dose AT administration in patients with severe coagulation disorder [57]. However, the three studies mentioned above were performed a long time ago, and very few studies have been conducted recently (https://clinicaltrials.gov/ct2/results?cond=trauma&term=antithrombin&cntry=&state=&city=&dist=). An adequately designed, randomized controlled study would be warranted to assess the efficacy of AT supplementation in TIC patients.

Newly Developed Recombinant Antithrombin

As described previously, the anticoagulant activity of AT increases by over a thousand-fold when it is bound to heparin or heparinoids. Some recombinant ATs have been synthesized but the limited biosimilarities hindered their penetration. Especially, the differences in the attached oligosaccharide side-chains abrogate the activity and shorten the half-life. In addition, heparin-binding capacity is tremendously affected by the core fucose at the reducing end of the attached oligosaccharides [58, 59]. It has been reported that fucosylation of the oligosaccharide at Asn 155 results in a reduction in the heparin-binding affinity of AT and that the oligosaccharide in human AT does not contain fucose. To produce a recombinant AT, fucosylation of the oligosaccharide was the major obstacle because the AT from animal cells is fucosylated [59, 60]. Recently, a recombinant AT possessing 4 oligosaccharides but lacking in fucose was produced by recombination (KW-3357, ACOALAN®, Kyowa-Kirin). A Chinese hamster ovary cell line was utilized to produce fucose-deficient AT [61, 62]. Approximately 95% of KW-3357 is composed of α-AT and its long half-life allows for a one-hour infusion daily. An RCT targeting resolution of DIC was performed in Japan, and the results demonstrated that KW-3357 had an effect equivalent to that of plasma-derived AT [63]. KW-3357 was approved in Japan in 2015 for the treatment of thrombosis in patients with congenital AT deficiency and DIC. Since the production of this type of agents is not limited by the amount of blood donated, they are expected as the therapeutics in the coming new era.

Conclusion

AT, the most important natural anticoagulant, plays a vital role in regulating over-activated coagulation and inflammation in critical conditions through multifactorial pathways. Since the activity of AT readily decreases via various mechanisms, its supplementation is expected to be useful in the treatment of TIC. Although high-quality evidence is still lacking, development of recombinant AT will open a new era of anticoagulant therapy.

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Physiology of Haemostasis: Plasmin-Antiplasmin System

5

Nicola J. Mutch and Claire S. Whyte

Fibrin as a Substrate

Plasmin is a potent trypsin-like serine protease that cleaves any substrate after lysyl or arginyl bonds. It activates growth factors and prohormones, actions that are outside the scope of this review, but its main substrate in vivo is fibrin. Many of the cleavage sites in fibrin have been revealed by the study of fibrinogen, which, as a soluble protein, is easier to analyse (reviewed by [1]). The ordered degradation pattern (Fig. 5.1) is detailed here as it is essential to our understanding of what is measured in assays of D-dimer and other fibrin degradation products (FDP). The first cut is to the α-chain of fibrinogen, releasing the αC fragments; the remainder of the molecule is called fragment X (~260 kDa). Fragment X is then cut in the α-, β- and γ-chains across the coiled coil that connects the central E and terminal D domains of fragment X. The cleavage occurs in two steps, first splitting the molecule asymmetrically to generate fragment Y (~160 kDa) and fragment D (~100 kDa) and then cleaving fragment Y into a second fragment D and fragment E (~60 kDa), which contains the amino terminal portion of all six polypeptide chains.

Newly formed fibrin is degraded by plasmin with the same cleavage pattern as fibrinogen, indicating that no major structural reorganization occurs during fibrin polymerization [2, 3]. In contrast, when fibrin is cross-linked by the transglutaminase factor XIIIa (Fig. 5.1, right), it is cleaved at a slower rate and different degradation products arise. D-dimer, which consists of two fragments D from adjacent fibrin monomers, cross-linked via their γ-chains remnants, is generated. This covalent dimer, bound non-covalently to fragment E, is the DD/E complex. This fragment also occurs in long arrays held together by uncleaved coiled coils [4]. Larger FDP have the capacity to reassociate with one another and with fibrin [5], so the substrate for fibrinolysis is not a single entity but a complex and dynamic one, in which both formation and degradation occur simultaneously. The clearance of FDP from the circulation is via the kidney and also liver, depending on the actual fragment [6, 7].

Fibrin as a Vital Surface for Plasmin Generation and Activity

Fibrin is at the heart of the lytic cascade and plays a vital role in "orchestrating its own destruction" [1]. This behaviour will be explained by considering the proteases and inhibitors that regulate

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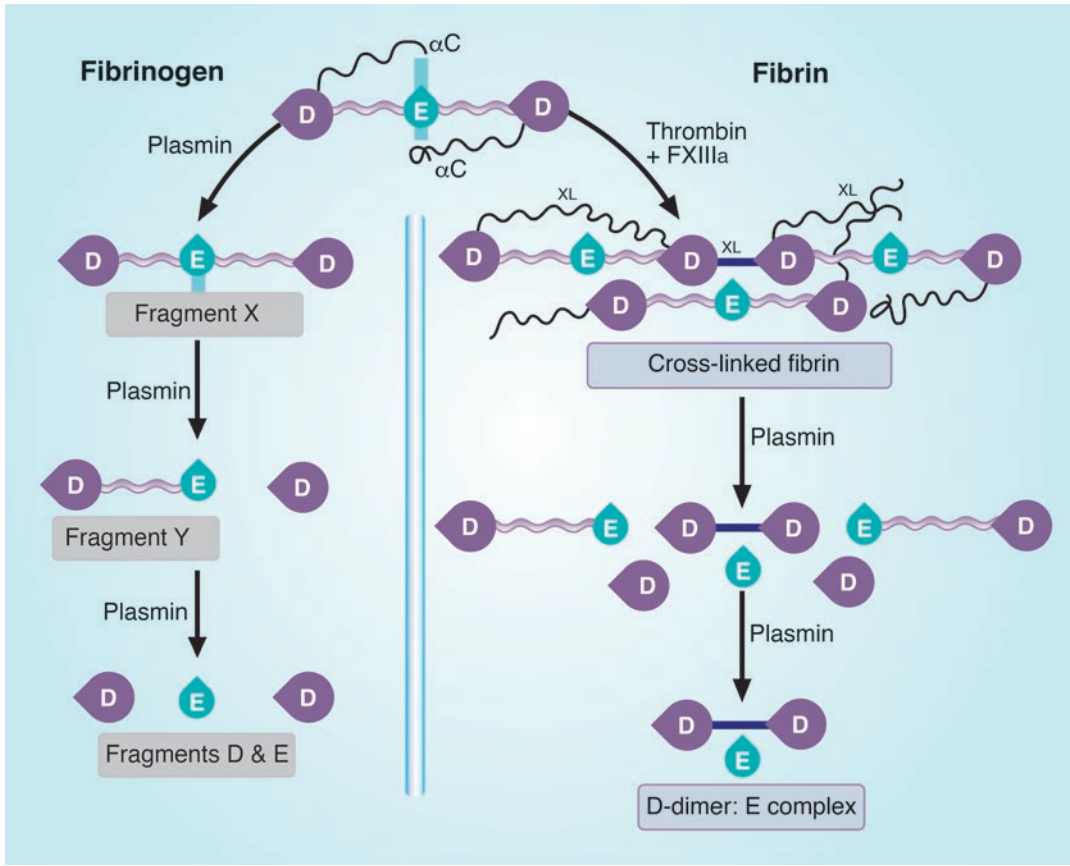


Fig. 5.1 Plasmin degradation of fibrin(ogen). Fibrinogen (**top**) is a three-domain globular protein with extending αC domains. Fibrinogen is degraded asymmetrically (**left panel**). Plasmin initially cleaves the αC domains from fibrinogen generating fragment X, which consists of all three domains connected by coiled coils, but lacks the Aα-chains and the Bβ1–42 sequence. The second cleavage occurs across the coiled coil that connects the central E and terminal D domains, generating fragment Y, which is composed of the central E domain connected by a coiled coil to the D domain. Fibrin is formed by cleavage of fibrinopeptide A and B from fibrinogen by thrombin (**right panel**). Thrombin also activates the transglutaminase factor XIII (FXIIIa) which cross-links (XL) fibrin longitudinally between the D domains and within the α-chain extensions. Cleavage of the two-stranded protofibrils by plasmin initially removes the cross-linked α-chains, followed by the coiled coils to liberate a series of fibrin degradation products (FDP), the smallest being DD/E. Larger complexes, such as DY/YD, are also released from cross-linked fibrin and are subsequently degraded to the DD/E moiety

the system, stressing throughout the governing role of fibrin.

Plasminogen

Plasminogen is a 92-kDa glycoprotein, abundant in plasma (Table 5.1). It is a classic zymogen, a single-chain molecule, activated by cleavage of one peptide bond to produce plasmin, in which the two chains are held together by two disulphide bonds. It is composed of several discretely folded domains. From the N-terminus, these are the activation peptide, a pan apple domain, kringles 1–5 and the protease domain (Fig. 5.2). The crystal structure of plasminogen indicates that two chloride ions in association with the pan apple and serine protease domain hold the zymogen in an inactive closed conformation [8]. The kringles, particularly kringle 1 [8], endow plasminogen

Table 5.1 Plasma balance of the principal proteins of the fibrinolytic system

Protein	Mr (kDa)	Molar concentration	Plasma $t_{1/2}$	Function
Plasminogen	92	2 μM	2.2 d	Zymogen
tPA	68	70 pM	4 min	Protease
scuPA	54	40 pM	7 min	Function
PAI-1	52	200 pM	8 min	Inhibitor
α_2-Antiplasmin	70	1 μM	3 d	Inhibitor
TAFI (pro-CpU)	60	75 nM	10 min[a]	Inhibitor
C1-inhibitor	105	1.7 μM	3 d	Inhibitor
α_2-Macroglobulin	725	3 μM	2–3 d	Inhibitor
PAI-2	46/70	<70 pM	–	Inhibitor
Factor XII	80	375 nM	2–3 d	Zymogen
Prekallikrein	88	450 nM	7–10 d	Zymogen
HMW kininogen	110	600 nM	9 h	Cofactor

[a]Activated form

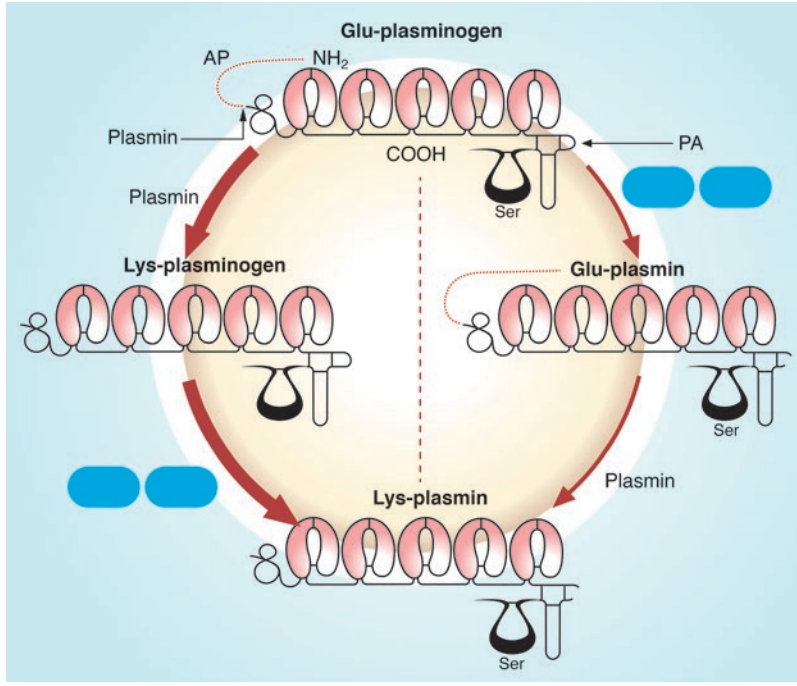


Fig. 5.2 Plasminogen activation. Plasminogen activator (PA) cleaves at Arg 561-Val 562, separating the B (light, protease or catalytic) and the A (heavy, kringle) chains. Glu-plasminogen and Glu-plasmin forms both contain the amino-terminal activation peptide from Gln1 to Lys 76 (shown in red). Plasmin can cleave this activation peptide (**left side**), generating Lys-plasminogen, an intermediate form that interacts with fibrin more efficiently and is more readily cleaved by tPA and uPA. It is this pathway of plasmin generation that occurs more readily, as indicated by the shading and heavyset arrows. Plasmin can also cleave the activation peptide from Glu-plasmin, generating Lys-plasmin (**right side**). The five kringle structures of the A-chain modulate binding of plasminogen to both fibrin and cell receptors. The catalytic centre contains the typical Ser-His-Asp residues and is the major site of interaction with its principal inhibitor, α2-antiplasmin

with the capacity to bind to cells and other proteins; the most relevant to this chapter are fibrin, α_2AP and TAFI. Such binding has profound effects on plasminogen activation. Plasminogen is primarily produced by the liver and is classified as an acute-phase protein [9]. Cells other than hepatocytes can produce plasminogen, for example, eosinophils, kidney, cornea, brain and adrenal medulla; such plasminogen is more likely to have local effects acting on substrates other than fibrin [10–13]. Human deficiency of plasminogen is uncommon, but when it occurs, it is often in association with fibrin deposition, for instance, in ligneous conjunctivitis [14].

Native plasminogen has several variants, in terms of limited proteolysis, degree of glycosylation and genetic polymorphism. For the purposes of this review, we will consider only the two main variants, Glu-plasminogen, the full-length form, and Lys-plasminogen, which has been processed to a variable extent at the N-terminus by trace plasmin. These two forms differ markedly in how efficiently they are activated (Fig. 5.2). Glu-plasminogen is a relatively closed structure [15], whereas Lys-plasminogen is more flexible and open; it binds to the plasminogen activator approximately tenfold more effectively [16–18]. Lys-plasminogen also binds to fibrin with higher affinity than Glu-plasminogen. The same is true of binding to plasminogen receptors, a group of proteins that are exposed on cell surfaces and bind to plasminogen via lysine residues [19]. Thus, through several mechanisms, Lys-plasminogen is activated more readily, especially on the fibrin or cell surface [20].

Plasminogen Activators

The principal plasminogen activators are tPA and uPA, while the contact pathway plays a role in some contexts. Activation of plasminogen is always by cleavage of Arg561-Val562 bond, yielding the two-chain active form, plasmin. It may be helpful to consider the life cycle of a plasminogen activator in terms of synthesis and release into the circulation, neutralization by inhibitors and clearance from the circulation by receptor-mediated mechanisms.

tPA is produced by endothelial and other cells as a single chain but is exceptional in that it is an active serine protease and not a true zymogen [21]. It circulates at low concentrations, mostly in complex with its primary inhibitor, PAI-1 [22, 23]. The plasma half-life is very short (Table 5.1) and shows a circadian rhythm, with lowest levels at night. Plasma tPA can be increased approximately fourfold under experimental conditions by venous occlusion or by drugs that induce acute endothelial release, such as bradykinin, histamine and β-adrenergic agents [24, 25]. Exercise also augments adrenalin-mediated tPA release, but also decreases clearance from the circulation [26]. Both tPA and tPA-PAI-1 complex are cleared by the low-density lipoprotein-related protein receptor (LRP) system [27].

tPA contains a finger domain and two kringle domains; the finger domain is the basis for its affinity to fibrin [28, 29]. This characteristic is crucial because tPA is a poor plasminogen activator in solution and requires fibrin to function as a cofactor in the reaction. Fibrinogen is not able to accelerate plasminogen activation by tPA, as the sites are encrypted in the precursor form [30]. Single-chain and two-chain tPA bind to fibrin in a comparable way [31] with plasminogen increasing the affinity of tPA for fibrin some 20-fold [32], as a result of ternary complex formation. In the absence of fibrin, the K_M values range from 9 to 100 μM plasminogen [33–35]. In most studies, this K_M value is three- to fourfold lower with two-chain tPA than with the single-chain form, a difference that essentially disappears in the presence of fibrin, when both forms of tPA yield K_M values ranging from 0.16 to 1.1 μM plasminogen [33, 35]. These concentrations are readily achieved in blood (Table 5.1). One clear reason for the experimental range in these data is that the kinetics are non-linear [34, 36, 37], with a dual-phase activation. Starting with Glu-plasminogen and tPA in the presence of fibrin, the initial K_M of 1.05 μM plasminogen was observed. Following plasmin formation and

generation of partially digested fibrin, binding of both plasminogen and tPA increased [38–42], so that the K_M was decreased to 0.07 μM plasminogen, with no change in k_{cat} [37].

uPA is synthesized by several cell types, particularly those with a fibroblast-like morphology, but also by epithelial cells [43], monocytes and macrophages [44, 45]. uPA can activate solution-phase plasminogen; it does not require fibrin as a cofactor. This behaviour, which is in marked contrast with tPA, is sometimes interpreted to suggest that uPA is unimportant in fibrinolysis and certainly it has roles in other processes, such as extracellular matrix degradation, cell migration, wound healing, inflammation, embryogenesis and invasion of tumour cells and metastasis [46, 47].

uPA has three domains: an epidermal growth factor (EGF) domain, a kringle and a protease domain. The uPA kringle has no affinity for fibrin. Its main binding, via the EGF domain located in the amino-terminal fragment, is with a specific uPA receptor, uPAR, described later in this chapter. uPA is expressed in its single-chain (sc) form, which has trace proteolytic activity; full activity requires cleavage of Lys158-Ile159 [48]. This can be achieved by several enzymes, the most relevant being plasmin [49, 50], factor XIIa and kallikrein [51]. Normal plasma contains scuPA at relatively stable concentrations of 2–4 ng per mL [52, 53] with little circadian fluctuation [54]. While endothelium is not a major source of uPA, there are reports of increased uPA following venous stasis [53], DDAVP infusion [55] and strenuous physical exercise [56], probably explained by decreased clearance from the circulation by receptor-mediated mechanisms. Under normal circumstances ,uPA activity is not detected in plasma, but both leukocyte-associated and free scuPA are elevated in leukaemia [57] and other disorders, including liver disease [58]. If generated, uPA is rapidly cleared from plasma, in a manner that depends on hepatic blood flow [59]. The LRP system binds and internalizes scuPA and uPA-PAI-1 complexes [27, 60, 61]. The asialoglycoprotein receptor, on parenchymal liver cells, also removes nonsialated uPA from the circulation [59].

Contact activation is a distinct process resulting from the interactions of four proteins, factor XII (FXII), prekallikrein (PK), factor XI (FXI) and high-molecular-weight kininogen (HK). Negatively charged surfaces such as polyphosphate [62, 63], RNA [64], misfolded proteins [65] and collagen [66] stimulate reciprocal activation of FXII to FXIIa and of PK to kallikrein (PKa) in association with its non-enzymatic cofactor, HK. The process is accelerated by zinc ions which induce a conformational change in FXII [67–71] and HK [72–74], thereby augmenting surface interactions. The downstream targets of these proteases have been debated as this pathway is associated with coagulation via cleavage of FXI to yield FXIa, inflammation by generation of bradykinin from HK and fibrinolysis.

Of note, while FXII is classified as a coagulation factor, it is structurally related to tPA, uPA and plasminogen [75, 76] and can function in plasminogen activation by different mechanisms. FXIIa directly activates plasminogen (Fig. 5.3) albeit relatively poorly compared to tPA and uPA [77–79]. However, the reaction is markedly enhanced by negatively charged surfaces such as dextran sulphate [80] and importantly by platelet-derived polyphosphate [81]. Circulating plasma concentrations of FXII are four orders of magnitude higher than tPA and uPA and, combined with the increase in plasma half-life, suggest that in certain environments or conditions, in vivo FXIIa could be a relevant plasminogen activator [82].

PKa generated by FXII-dependent [51, 83] and FXII-independent [84] pathways is a kinetically favourable activator of scuPA (Fig. 5.3) which in turn activates plasminogen. Finally, the vasoactive peptide bradykinin, described above in the inflammatory arm of the contact pathway, also indirectly impacts fibrinolysis by stimulating tPA release from endothelial cells [85, 86]. These three functionally distinct mechanisms implicate the contact pathway as a modulator of plasminogen activation, but further studies are necessary to unravel its contribution in different milieu.

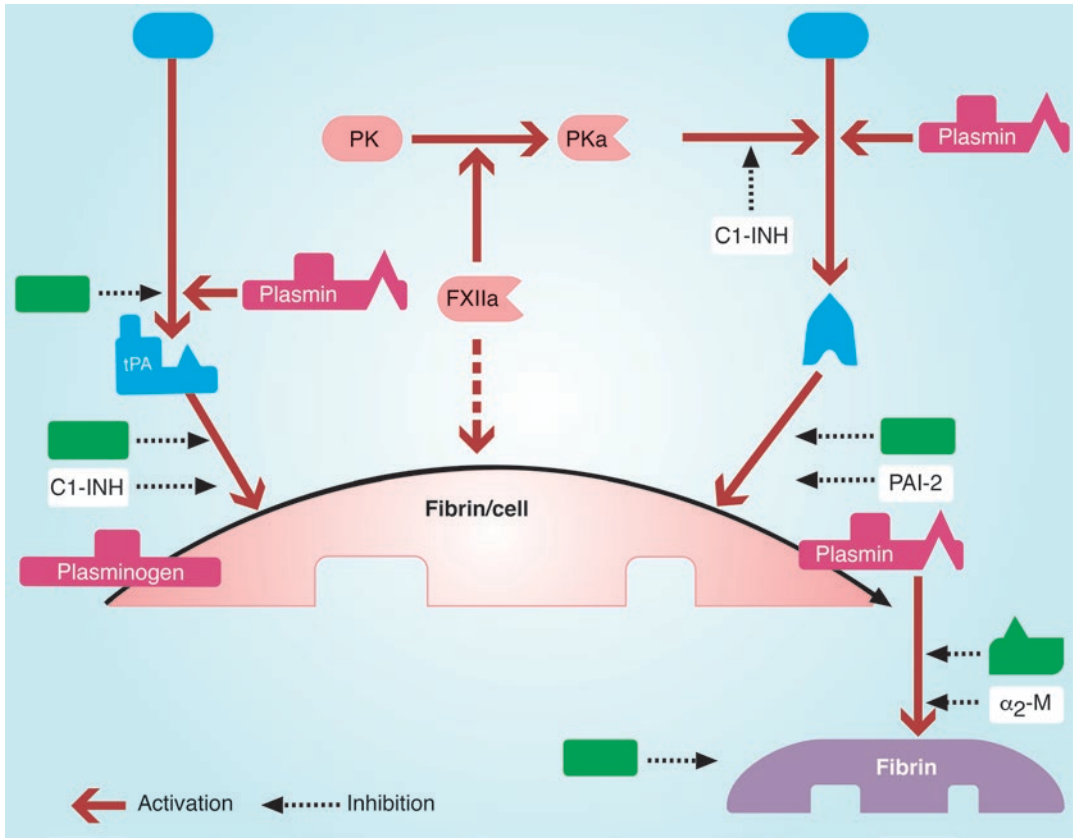


Fig. 5.3 Significant players in the fibrinolytic system. scuPA, single-chain urokinase plasminogen activator; sctPA, single-chain tissue plasminogen activator; α_2AP, α_2-antiplasmin; α_2M, α_2-macroglobulin; C1-INH, C1-inhibitor; PAI-1, plasminogen activator inhibitor 1; PAI-2, plasminogen activator inhibitor 2; TAFI, thrombin-activatable fibrinolysis inhibitor; FXIIa, activated factor XII; PK, prekallikrein; PKa, kallikrein. Activation of plasminogen to plasmin usually occurs on a surface, either fibrin or a cell membrane. Once formed, plasmin degrades fibrin as described in Fig. 5.1

Inhibitors of Plasmin Generation and Activity

The proteases of the system are controlled by inhibitors, most of which act directly on the proteases and form inactive complexes with them. PAI-1 and α_2AP are members of the serpin family, which inhibit plasminogen activators and plasmin, respectively, via a reactive centre loop that mimics the protease substrate (reviewed by [87]). A second mode of action, exemplified by TAFIa, is modulation of the generation of fibrinolytic activity.

PAI-1 is the principal inhibitor of tPA and uPA and inhibits both with second-order rate constants greater than 10^7 M^{-1} S^{-1}, close to the diffusion limit [88]. It does not inhibit scuPA, which is largely inactive, but it does associate with scuPA non-covalently [89]. It is an unusual serpin in that it spontaneously loses activity by insertion of its reactive centre loop into the core of the molecule [90]. This inactive form was originally termed "latent", which unfortunately gives an impression that the latent material is physiologically activated. Reactivation is indeed possible, but only by chemical denaturation and

A L G r a w a n y

refolding [91]. It was characterized originally as a product of endothelial cells but it is synthesized by most cells in culture, including megakaryocytes [92], endothelial cells [93], hepatocytes [94] and adipocytes [95–97]. PAI-1 is synthesized in its active form and circulates in plasma in complex with vitronectin, which stabilizes the active form substantially lengthening its plasma half-life [98].

PAI-1 plasma concentrations are approximately 20 ng per mL [99–101] but reported values range, even in normal individuals, from barely detectable to 40 ng per mL. The variations may be circadian; PAI-1 plasma concentration peaks in the morning [102–104], and in addition, PAI-1 is an acute-phase protein [105]. Understanding its behaviour in response to stress is complicated by the fact that it is synthesized by a wider range of cells than the classic acute-phase proteins and that it is responsive to many stimuli. Some variations in PAI-1 measurements may be methodological. It is necessary to exclude platelets and their release products in analysis of plasma PAI-1, since platelets are the major pool (more than 95%) of circulating PAI-1 antigen [106]. PAI-1 in plasma is in excess over tPA (Table 5.1); therefore, most of the tPA is in complex with PAI-1. Immunological assays of either protein generally measure both free and complexed forms, requiring care in interpretation. Gram-negative septicaemic patients have dramatically elevated plasma PAI-1 concentrations, as much as 50-fold over normal, and are associated with high mortality [107]. High circulating PAI-1 is associated with a range of disease, including cardiovascular disease [108, 109] and cancer [110]. The causal significance remains unclear, and it seems that high PAI-1 does not independently predict disease when factors like obesity, diabetes and elevated triglycerides are taken into account [111]. There is a guanine insertion/deletion polymorphism at position 675 in the PAI-1 promoter [112], which is associated with differences in circulating PAI-1 [113], but the predictive power of this polymorphism appears to be low [111, 114]. Deficiency of PAI-1 in humans is rare but it causes a lifelong bleeding disorder, characteristically after a delay, consistent with normal clotting but premature lysis of haemostatic plugs at sites of vascular trauma [115–118]. Fibrinolytic inhibitors such as tranexamic acid decrease plasminogen activation and therefore are effective in normalizing haemostatic function in such patients [117, 118].

α_2AP is the principal inhibitor of plasmin, the term fast-acting being used to stress the rapid inhibition, with a second-order rate constant of 4×10^7 per M per second [119]. Its plasma concentration is 1 μM, about half the molar concentration of plasminogen; it has to be remembered that plasma plasminogen is seldom, if ever, entirely converted to plasmin, so the inhibitor is usually in excess. It is synthesized in the liver and consequently decreased in patients with advanced impairment of hepatic function. The $t_{1/2}$ of the native inhibitor is approximately 3 days, whereas the covalent plasmin/α_2AP (PAP) complex is cleared with a $t_{1/2}$ of approximately 0.5 days [120].

α_2AP circulates in several forms, depending on limited proteolysis at N- and C-termini. The processing of the inhibitor has little impact on the inhibitory capacity of α_2AP which depends on the reactive centre loop. Newly produced α_2AP (Met form) has 12 residues at the N-terminus that can be cleaved to yield N-terminal Asn [121] by an antiplasmin cleaving enzyme (APCE) [122]. Both forms are equally represented in plasma [123]. The N-terminal cleavage is important because it reveals Gln2, in the processed, Asn form, the Gln2 being cross-linked to Lys 303 of the fibrin(ogen) Aα-chain by FXIIIa [124, 125]. In contrast, in the Met form, Gln2 is blocked [126]. Fibrin to which α_2AP is cross-linked resists lysis by plasmin, and this observation was central to the discovery of the first human deficiency of α_2AP [124]. Consistent with this, antibodies that react specifically with cross-linked α_2AP stimulate lysis of fibrin [127].

Comparison of α_2AP with other members of the serpin family reveals that it has a C-terminal extension of some 50 residues [121]. This full-length form and a shortened form are both detectable in normal human plasma [128]. The full-length form binds plasminogen but the processed form, which is still a potent inhibitor of plasmin, cannot bind plasminogen [129].

The enzyme responsible for this C-terminal cleavage has not yet been characterized. The ratio of two forms, plasminogen binding to non-binding, is approximately 2:1 in plasma. This was still true even in advanced liver cirrhosis [58], despite the impaired synthesis of α_2AP in these patients.

Binding of α_2AP to plasminogen competes with the plasminogen-fibrin interaction, as it occurs via the same lysine binding site (Fig. 5.4). Plasmin formed on fibrin is therefore relatively protected from the action of α_2AP [130], a key finding in the control of fibrinolysis [130]. The experimental basis for this concept used lysine analogues, in the presence of which α_2AP was about 100 times less effective in inhibiting plasmin [119]. The exact Lys residues responsible for binding the C-terminal region of α_2AP to plasminogen are not conclusively defined. Studies have shown a major effect of Lys452, but that other internal Lys residues "tether" the kringles [131, 132]. A different study, in which Lys residues were systematically mutated, suggested that Lys436 had the greatest effect [133].

Thrombin-activatable fibrinolysis inhibitor (TAFIa; also known as carboxypeptidase B, U, R [134] and *CPB2* gene product [135]) removes C-terminal lysyl residues from fibrin, which, as previously stressed, are important in the binding of plasminogen [136]. TAFI is produced as a zymogen (or procarboxypeptidase) and is activated by the thrombin/thrombomodulin complex [137] or by plasmin in the presence of glycosaminoglycans [138]. Its activation by thrombin makes it an important molecular link between fibrinolysis and coagulation [139]. TAFI is produced in the liver but there is considerable variation in normal circulating concentrations [136, 140] and only a fraction need be activated for full physiological impact [137]. Its activity is controlled by its instability, with an effective plasma half-life of only about 10 min [141]. The function of TAFIa was shown in clot lysis assays; potato tuber carboxy-

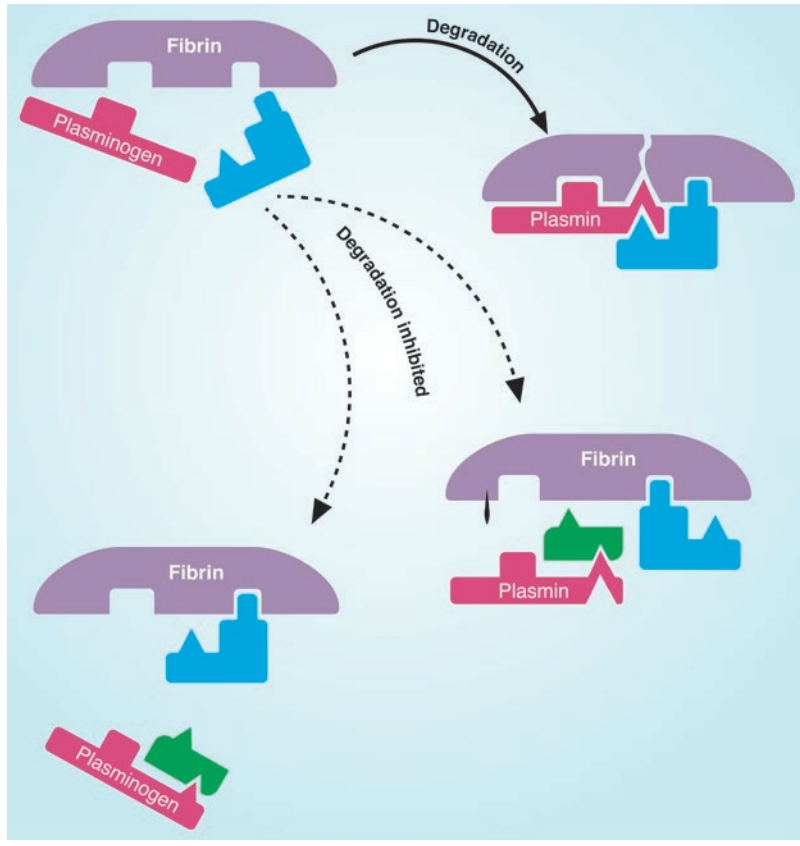


Fig. 5.4 Localization of plasmin(ogen) and tPA on fibrin; interference by α2-antiplasmin. Fibrin binds plasmin(ogen) and tPA directly and acts as a cofactor in plasminogen activation, thereby augmenting its own destruction. α_2-Antiplasmin (α_2AP) can bind to plasmin(ogen) in solution, obstructing binding to fibrin. α_2AP is also cross-linked to fibrin, via the action of factor XIIIa, which prevents plasmin from binding to fibrin and neutralizes the plasmin activity. These events hamper plasminogen activation on fibrin and/or inhibit plasmin activity, thereby limiting fibrin degradation

peptidase inhibitor relieves the inhibition [139, 142]. This approach and more sensitive and specific assays for TAFIa have shown that the carboxypeptidase must be maintained at a threshold level to be effective in modulating fibrinolysis; this level fluctuates in relation to plasmin concentration [143]. Several polymorphisms in the TAFI gene have been reported, resulting in four isoforms [144, 145]. These isoforms explain the normal wide range in concentration, but do not correlate strongly with disease [145, 146]. Thr325Ile polymorphism has been shown to be an independent risk factor for ST acute myocardial infarction in a Mexican population [147]. Elevated TAFI appears to be a mild risk factor for venous thrombosis [148], and it also increases in inflammation, correlating with other acute-phase markers [149]. Contrary to this, patients recently suffering a myocardial infarction have been shown to have lower levels of TAFI [150].

Increased fibrinolytic activity in haemophilia patients is explained by defective TAFI activation. Most thrombin is formed after clot formation, mainly by back activation of FXI by thrombin, with deficiencies in FXI resulting in a mild to moderate tissue-specific bleeding disorder (haemophilia C). In the absence of FXI, clots lyse more readily [151], which is associated with the loss in feedback activation of FXI by thrombin [152]. The enhanced generation of thrombin augments TAFI activation stabilizing clots against premature lysis [153, 154]. In line with this, defective TAFI activation in congenital haemophilia A is associated with uPA-mediated joint bleeding [155]. Addition of TAFI, thrombomodulin or factor VIII to haemophilia A plasma restores normal fibrinolysis [156]. Consistent with this, incorporation of anti-factor XI antibodies or inhibition of TAFIa in a rabbit model resulted in an almost twofold increase in endogenous thrombolytic activity [157].

We described earlier the potential contribution of the contact pathway in facilitating plasminogen activation. The role of FXIa in sustaining thrombin generation and therefore TAFI activation implicates the contact pathway in antifibrinolytic as well as profibrinolytic mechanisms. Indeed, abnormal clot structure and sensitivity to fibrinolysis have been described to help predict the risk of bleeding tendency in severe and partial FXI deficiency [158, 159].

Other Inhibitors

In most situations, α_2AP, PAI-1 and TAFI are the major gatekeepers in the regulation of plasmin generation and activity, but there are other inhibitors that may function in specific circumstances, which will now be introduced briefly.

PAI-2 is an inhibitor of uPA purified from human placenta and the cell line U-937 [160, 161]. The role of PAI-2 as a PA inhibitor has been questioned [162], as mice deficient in PAI-2 do not present any major haemostatic abnormalities [163]. The intracellular location of this serpin and the fact that it is a much poorer inhibitor of uPA and tPA [161] have led researchers to believe that its functions may lie outside the haemostatic cascade. In the circulation, monocytes are the main reservoir of PAI-2 [164] and may increase fibrin stability on migration into thrombi, particularly as PAI-2 is cross-linked to fibrin [165]. Interestingly, deficiency of PAI-2 is found to interfere with venous thrombus resolution in mice [166], most likely due to the instigated inflammatory response. PAI-2 is not normally detected in normal plasma, except in pregnancy, where it rises steadily to reach approximately 250 ng/mL by the third trimester [167]. In placental dysfunction[413,414] and intrauterine growth retardation [168–170], the rise in plasma PAI-2 is much smaller, highlighting its importance in normal foetal development. PAI-2 also occurs in plasma of patients with acute myeloblastic leukaemia (M_4 and M_5, [171]) and in patients with sepsis [172]. Local PAI-2 activity appears to be relevant to a number of cancers, and studies on the function of this serpin in these settings may provide further clues to its true biological role [173, 174].

α_2-Macroglobulin (α_2M) is a non-serpin inhibitor of wide specificity. This breadth of targets and its relatively high plasma concentration (2.5 g per L, 3 μM) make it an effective stand-in inhibitor that contains the activity of many proteases, including plasmin, tPA and uPA [57]. α_2M

is a tetramer made up of a pair of dimers containing two reactive sites. When proteases are inhibited by α_2M, they generally retain activity towards small peptide substrates, but are unable to cleave larger targets.

C1-inhibitor is a highly glycosylated serpin that directly modulates the activity of C1r and C1s proteases of complement C1. It also inhibits the contact proteases, FXIIa, FXIa and PKa, as well as tPA, plasmin and uPA. It circulates in plasma at a relatively high concentration (1.7 μM). When tPA is in excess over PAI-1, complex formation with C1-inhibitor is observed [23, 58, 175]. Its diverse targets suggest that it would function in regulating contact phase–dependent fibrinolysis and the conversion of scuPA to uPA (Fig. 5.3). Indeed, peripheral blood mononuclear cells from patients with hereditary angioedema (HAE), arising from a deficiency in C1-inhibitor, express elevated levels of uPAR [176]. HAE is also associated with aberrant fibrinolytic activity as a result of dysregulated plasmin generation and inhibition. Indeed, during activation of fibrinolysis, approximately 15% of plasmin inhibition is reportedly accounted for by C1-inhibitor [177]. The increase in bradykinin generation in HAE patients will also augment tPA release from the endothelium.

Regulation of Plasmin Generation and Activity

So far, we have highlighted three important concepts: zymogen activation, protease inhibition and, crucially, the role of fibrin in promoting activation of plasminogen and protecting plasmin from inhibition. Further discussion requires consideration of particular situations, so we will now examine the balance of the various proteases and inhibitors in plasma, on platelets, cell surfaces and thrombi.

Plasma Balance

Plasminogen, the central player of the fibrinolytic system, circulates at approximately 5 orders of magnitude higher than tPA and scuPA (Table 5.1). Plasminogen is turned over relatively slowly, with a half-life of 2.2 days for Glu-plasminogen and 0.8 days for Lys-plasminogen, while tPA and scuPA have plasma half-lives of only minutes. From this we can infer that the rates of synthesis, release and clearance are low for plasminogen and much higher for the PA, illustrating the more dynamic part of the system. Similar considerations apply to the main inhibitors. PAI-1 is present in plasma at only 400 nM, while α_2AP circulates at 1 μM, and again the plasma half-lives are in marked contrast.

Fibrinolytic activity is not normally detectable in plasma because plasminogen is a true zymogen, and therefore inactive, while the one active PA in plasma, tPA, is normally controlled by an excess of PAI-1. Even if the concentration of PAI-1 were insufficient for full neutralization, then α_2AP, C1-inhibitor and α_2M would act as backup inhibitors. The other potential activator, scuPA, is not sufficiently active to initiate the process of plasminogen activation, as prior activation by plasmin or kallikrein is necessary. Any trace of plasmin generated in plasma would be quickly neutralized by α_2AP, again endorsed as necessary by other inhibitors, especially α_2M. So the quiescence of the system, in plasma, is maintained by tight control of protease activity, both at the level of existence of plasminogen as a zymogen and at the level of control by inhibitors, primarily PAI-1 and α_2AP.

Cellular and Platelet Contributions

While the central role of fibrin in controlling activation of plasminogen and protection of plasmin has been appreciated for several years [130], we are now aware that many of the same general characteristics apply to cell-based or platelet-based fibrinolysis. That is, more efficient activation of plasminogen occurs on the surface of cells, while cell-bound plasmin is protected from inhibition by α_2AP [178]. Plasminogen binding to circulating cells, including monocytes, neutrophils and platelets, was first reported in 1985 [179]. Binding to platelets is now known to occur

in distinct locations, dependent on the specific subpopulation [180]. The proteins responsible for binding vary from cell to cell but include $PlgR_{KT}$, α-enolase, S100A10 (functioning with annexin A2), actin, cytokeratin 8 and integrins $\alpha_{IIb}\beta_3$ and $\alpha_M\beta_2$ (reviewed by [181]). The binding of plasminogen to these receptors tends to be low affinity but high capacity, with some cell-surface proteins only found on cells undergoing apoptosis. Not all these proteins express the C-terminal Lys that is expected of a plasminogen-binding protein. $PlgR_{KT}$ is a true membrane protein and is synthesized with a C-terminal Lys residue. tPA binding to cells occurs via annexin II and also directly to $PlgR_{KT}$ [181]. Other reports on tPA receptors, which have been characterized in less detail [182, 183], may be of the same or similar molecules.

uPA and scuPA bind to a well-characterized receptor, uPAR (CD87), with high affinity (K_D 10^{-9} to 10^{-11} M) depending on the cell type [184]. uPAR is not a transmembrane protein but is attached to membranes via a glycosylphosphatidylinositol (GPI) anchor. Binding of uPA to uPAR elicits signalling [185], via other intracellular proteins. Other proteins also bind uPAR, including vitronectin and integrins in complex with caveolin [186]. The uPA/uPAR complex on some cells is associated with Endo 180, also known as uPAR-associated protein (uPARAP) [187], and has a role in collagen IV internalization [188]. uPA bound to uPAR is still inactivated by PAI-1 but not as fast as in solution [189]. The uPA/PAI-1 complex is then internalized, while uPAR is recycled to the surface, a process that also involves LRP [61, 190]. uPAR has clear roles in migration and metastasis. In terms of fibrin degradation, we must distinguish between activation by scuPA and uPA. In the case of uPA, which can freely activate plasminogen in solution, binding to uPAR seems not to affect plasminogen activation, but the activity of scuPA is increased by two orders of magnitude when it is bound to uPAR on the surface of monocytes [191, 192]. An elegant experiment in which a uPA variant was directly anchored to the cell surface showed a stimulation of plasminogen activation similar to that achieved by binding to uPAR [193]. This is consistent with the principal function of uPAR being one of localization of uPA to the cell surface rather than enhancement of catalytic activity. The same co-localization and reciprocal activation of scuPA and plasminogen occurs on platelets [50], which do not express uPAR, indicating there are additional receptors yet to be discovered. Other studies show that cellular binding of plasminogen and (sc)uPA does not have to be on the same cells or surface to facilitate fibrinolysis [194].

Platelets make several contributions to clot stability and lysis. On the profibrinolytic side, activated platelets exhibit endogenous plasmin activity [180] and surface-bound plasmin, formed from local plasminogen, is afforded protection from inhibition by α_2AP. On the antifibrinolytic side, there is the physical barrier to lysis that results from clot retraction, added to which platelets have a pool of FXIII [195] that stabilizes fibrin. Further, platelets are a source of the three main inhibitors of fibrinolysis, PAI-1, α_2AP and TAFI (Fig. 5.5). These platelet-derived pools result from synthesis and packaging of the inhibitors at the megakaryocyte stage. Indeed, it has been reported that while platelets are devoid of a nucleus, they are capable of synthesizing large quantities of PAI-1 [196]. Recent work has illustrated that despite our traditional view that PAI-1 is released from platelets, a considerable amount of active PAI-1 is retained on the activated platelet membrane [197]. In terms of activity, platelet PAI-1 is less active than plasma PAI-1, but platelets still account for some 50% of the total circulating active PAI-1. The platelet pools of α_2AP and TAFI are not as substantial, accounting for less than 1% of the total blood pool [198, 199] and may have functional significance in particular niches. The interaction of platelets and fibrin is regulated by the integrin αIIbβ3 and is key to the process of clot retraction. A recent elegant study has illustrated that the processes of clot retraction and fibrinolysis are mechanistically coupled indicating their intrinsic interaction in vivo to modulate thrombus size [200].

Studies on human thrombi reveal that the inhibitors of fibrinolysis, especially PAI-1, accumulate in great excess over proteases [201, 202],

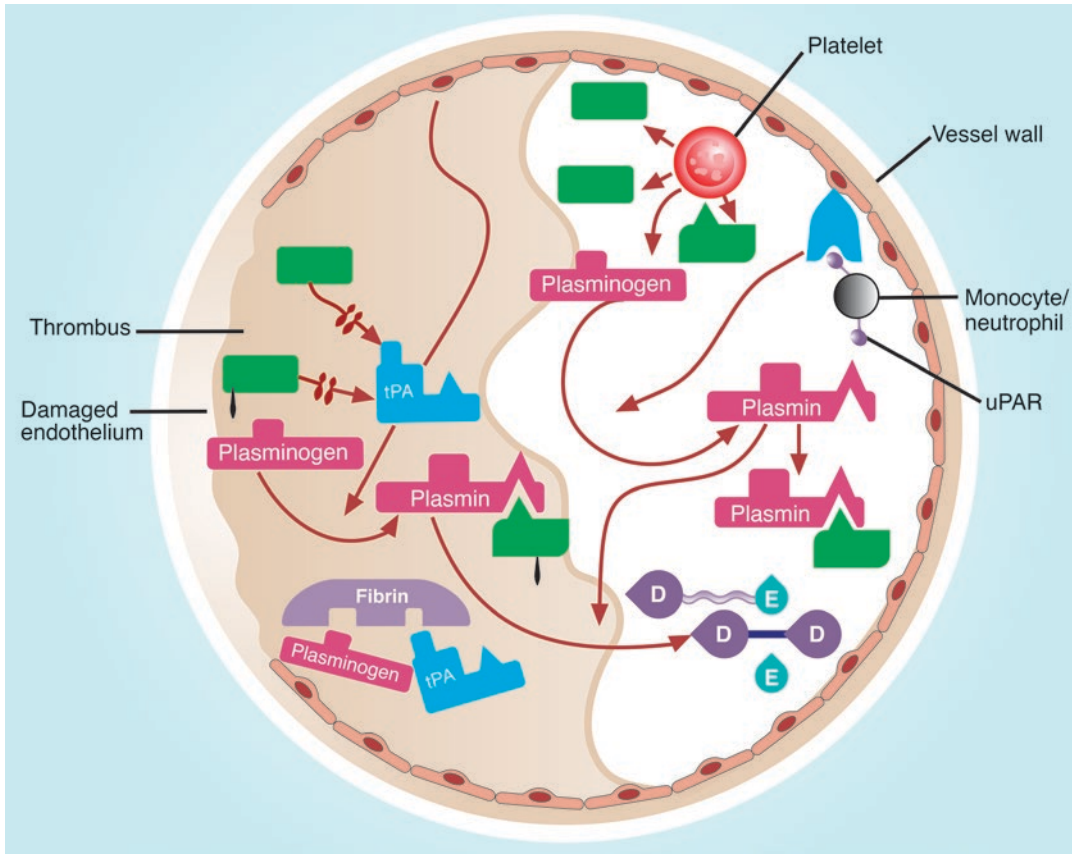


Fig. 5.5 The balance of fibrinolysis in the injured vessel wall. Schematic representation of the different modes of plasmin formation, inhibition and clearance within a damaged vessel wall, with a partially occluding thrombus. Solution, cell-surface and fibrin phases of plasmin formation are represented. Plasminogen circulates at a relatively high concentration (2 μM) and is readily incorporated into a forming thrombus by virtue of its fibrin binding capacity. tPA is largely derived from the endothelium and only circulates at low concentrations with high turnover. tPA-mediated plasminogen activation is slow in solution, but is enhanced severalfold when bound to its cofactor, fibrin. uPA is found in the circulation and is from monocytes and neutrophils. uPA does not exhibit fibrin specificity and readily activates plasminogen in solution while bound to its cell surface receptor, uPAR. Association of uPA with uPAR provides a focal point for plasmin generation. Plasmin degrades fibrin into fibrin degradation products, represented here as D dimer and DD/E complexes. The system is regulated by several inhibitors; only the principal ones are shown here for clarity. Complexes of active enzyme and inhibitor are rapidly cleared from the circulation via a low-density lipoprotein receptor. PAI-1 circulates at low concentrations, but a large pool is released from platelets upon activation. PAI-1 can inhibit tPA and uPA in solution and in the presence of fibrin or cell surfaces, but this latter process is generally less efficient. α_2-antiplasmin (α_2AP) is abundant in plasma and a minor pool is also released from activated platelets. α_2AP inhibits plasmin generation in solution, but plasmin formed on cell or fibrin surfaces is relatively protected. α_2AP is cross-linked directly onto fibrin localizing it at the site of plasmin generation. TAFI is found in plasma and platelets and can be cross-linked to fibrin. TAFI is activated by the thrombin/thrombomodulin complex or plasmin to generate TAFIa which downregulates plasminogen activation on fibrin, by removing the C-terminal lysine residues that are important for the binding of plasminogen to fibrin. This intricate sequence of events and interactions modulate fibrin accumulation in the body in a precise and coordinated manner. The many different feedback loops and surfaces involved localize reactions thereby preventing excessive plasmin generation in the circulation

providing an explanation as to why established thrombi are often resistant to lysis. Observations on human thrombi also show they retain substantial amounts of coagulant and fibrinolytic activity [203, 204]. Of course, such diverse material is taxing to work on in a quantitative way and there are obviously differences in venous and arterial thrombi and between mural and luminal thrombi.

Our studies in Chandler model thrombi showed that these thrombi lyse spontaneously, with fibrinolytic activity that could be ascribed primarily to uPA but to a lesser extent to tPA, elastase and cathepsin G [205]. This spontaneous generation of fibrinolytic activity [204] was dependent on polymorphonuclear cells, primarily neutrophils, generating local uPA activity on uPAR [205]. Plasma α_1-antitrypsin was crucial in protecting the activity from neutrophil elastase [206]. The integrin $\alpha_M\beta_2$ is important in the generation of such local activity [207]. Discovery of a role for local uPA in thrombus lysis ran counter to the usual proposition that tPA's role is fibrin degradation and uPA mediates other cellular events. There is, however, compelling support for it from a number of other studies, including failure of thrombi from uPA gene knockout mice to resolve [208]. In that model, the uPA activity was associated primarily with monocytes, which migrate into thrombi [209] and express fibrinolytic activity [44]. Indeed, monocyte-bound uPA has been shown to reduce thrombus size in a model of venous thrombosis [210].

Questions That Remain

What Initiates Fibrinolysis?

The available evidence suggests that the Glu-plasminogen to Lys-plasminogen conversion is the initiating event. It has the required features of leading to large-scale amplification as the plasminogen binding sites on fibrin are revealed by partial lysis, and formed plasmin is protected from α_2AP. Part of the same question is which PA is responsible for the first molecules of plasmin that allow Glu-plasminogen to be converted to Lys-plasminogen? In the context of fibrin, with no cells or platelets, it may be tPA, a few molecules of which may be free of PAI-1, that provides initiation, especially since its single-chain form is active and not as readily inactivated by PAI-1. This has been suggested by Thorsen (1992) in his well-established biphasic lysis [211], where a small amount of plasminogen on fibrin fibres is activated and then degrades fibrin to generate C-terminal lysine residues that bind additional plasminogen and perhaps tPA, leading to the second faster phase of tPA-mediated fibrinolysis [212]. The molecular interactions and specific binding sites involved have been extensively reviewed [30]. Experiments using tPA variants show that the finger domain of tPA plays a more dominant role in the interaction with fibrin than the kringle 2 interaction with C-terminal lysine residues [213]. This suggests that it is the binding of plasminogen to partially degraded fibrin, and thus subsequently the opening of the closed to open confirmation, that is the crucial step in the rapid second phase of fibrinolysis. This central role of plasminogen may suggest that the PA responsible for activation is less crucial than previously assumed. Our experiments with TAFI demonstrated a similar delay in lysis regardless of the PA used [214] and we interpreted this as plasminogen primarily controlling fibrin-bound plasmin generation.

If a cell membrane is present, then it may be scuPA, bound to cellular uPAR or on platelets, that yields the initial protease activity. This is suggested on the basis of several experimental systems, including data showing that the ordered addition of scuPA and then tPA [215] is potentially more effective than either agent alone. Our own work on Chandler model thrombi underlines the importance of the scuPA/uPA system in spontaneous lysis [205] but affirms the involvement of other proteases, especially tPA [204]. When in association with cellular uPAR, scuPA binds PAI-1 and other serpins reversibly [89]. This has been interpreted in terms of receptor-bound scuPA initiating proteolytic activity, with conversion to uPA achieving inhibition thereby regulating the activity [216].

How Best to Measure Fibrinolysis?

Fibrinolysis, like other cascade systems, coagulation and complement, can be studied by various means. Individual components can be quantified, either as antigen or activity, and under defined situations can provide clear answers. However, the complexities of the system mean that a change

in one factor can influence measurements of another and therefore it is important to interpret results with caution. As an example, tPA activity is challenging to measure in plasma, as it is at the limit of detection of most assays. Elevated PAI-1 may depress the activity that is measured. Frequently, a manipulation of plasma is necessary to reveal tPA activity, including acidification of plasma or preparation of a euglobulin fraction, where tPA, plasminogen and fibrinogen are retained. Most inhibitors are removed but about 50% of PAI-1 is retained [100], and these facts must be borne in mind for valid interpretation. As mentioned previously, circulating tPA is variable, whether at the level of synthesis or release; therefore, it is vital to consider the time course as each sample represents a snapshot. Rapid hepatic clearance of tPA and of tPA-PAI-1 complex from the circulation rapidly restores the system to normality, allowing key events to be overlooked.

It is often essential to measure more than one analyte for a fuller appreciation of the system. Ideally, the aim is to know how much enzyme is free and/or active and how much has been converted to a complex, such as tPA-PAI-1. A combination of ELISA and activity assays may provide a clear picture, but only if the specificity of the ELISA is known in some detail. Ideally, measurement of PA would be complemented by examining a consequence of the elevation, for instance, the fibrin degradation products produced, which of course reflects the presence of the fibrin substrate, or generation of the plasmin-α_2AP complex. The essential feature of ELISA for a complex is the use of antibody to one of the proteins, e.g. α_2AP, as a capture system and an antibody to the second moiety, e.g. plasmin, in the detection system. The capture antibody in this example will bind free α_2AP and α_2AP in complex, giving rise to potential competition and misrepresentation of the results. This element limits the use of these assays to situations where the free protein is decreased, for instance, in liver disease, where α_2AP is lower than normal. Other approaches to measuring overall fibrinolytic activity in plasma include measurement of a zone of lysis on a fibrin plate, clot lysis assays and zymography. Recently, a method which combines magnetic immunocapture of leukocyte-derived microvesicles and chromogenic measurement of plasmin generation has been described [217]. These can all be useful but there are limitations associated with most individual assays. For instance, in plasma clot lysis assays, the effects of FXIIIa cannot be reproducibly observed [218]. In addition, the overwhelming effects of α_2AP make it difficult to see inhibition by PAI-1. Failure to be alert to such considerations gives rise, in the literature, to many inappropriate interpretations about the relative importance of particular proteases or inhibitors. In all assays, the balance of enzyme to inhibitor ought to be as close to physiological as possible. When tPA is added, it should be at a low concentration, always remembering that it is a catalyst, not a reagent that is consumed. The literature abounds with examples where PA are added at high concentrations, simply to speed up the assay. This distorts a system that is designed to be delicately poised and generates artefacts of the experimental system rather than a true reflection of what goes on in vivo.

Detailed analysis of the fibrinolytic system is only practical for small numbers of samples, but, for large clinical cohorts, the aim is to obtain an insight from a limited number of assays. Not surprisingly this has promoted the use of overall measures of activity, such as global assays of fibrinolysis, which have inherent advantages and some limitations. Thromboelastography is rapid, widely available and easily applicable to large sample sizes. However, most studies add tPA as a stimulus. In this situation, added enzyme should be kept to a minimum, to avoid generating results that are far from physiological. Another global assay quantified fibrin degradation products after collection of blood samples onto thrombin [219]. Comparison of samples with and without aprotinin gives a measure of global fibrinolytic capacity, an approach that has proved useful clinically [220]. It should be noted that thrombin greatly enhances endogenous fibrinolytic activity, probably by inactivation of PAI-1 among other mechanisms. This consideration serves as a useful aide-mémoire that fibrinolysis is not an independent system. As Ratnoff reminds us, “The coagu-

lation, fibrinolysis, complement and kinin pathways are studied separately by scientists for their convenience. In life, they form a seamless web" [221]. Undoubtedly, we choose our approaches and molecules of interest to us, and may well ignore other players, by virtue of the experimental system used. These choices may be convenient, but we must bear in mind the selection bias introduced into the system.

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Part III

Pathophysiology of Trauma Induced Coagulopathy

Thrombin Formation

6

Beth A. Bouchard and Kalev Freeman

Thrombin Activation Controls Fibrin Deposition

Thrombin (factor IIa) is the ultimate protease in the clotting cascade, and the enzymatic steps of prothrombin cleavage leading to thrombin activation limit the rate of the coagulation process. Thrombin catalyzes fibrin formation and plays a key regulatory role in determining flux through the cascade (Fig. 6.1). Even slight deviations in the balance defining essential and pathologic thrombin generation result in bleeding and thrombotic disorders, demonstrating the critical significance of thrombin in hemorrhage control.

In the cell-based model of coagulation, enzymatic reactions occur in three stages of thrombin generation regulated by cell surfaces: initiation, amplification, and propagation [1]. Initiation occurs after damage to the vascular endothelial lining exposes subendothelial tissue factor to flowing blood. Exposed tissue factor binds to subnanomolar concentrations of plasma factor VIIa forming the extrinsic tenase complex and catalyzing the formation of factor Xa and factor IXa. Low levels of thrombin formed by factor Xa (insufficient to cleave fibrinogen) catalyze the proteolytic activation of factors XI, V, and VIII as well as platelets to greatly amplify flux through the cascade. Factor IXa functions as the proteinase in the intrinsic Xase complex, and in conjunction with the nonenzymatic cofactor, factor VIIIa bound to platelets adhered and activated at sites of injury also activates factor X to factor Xa.

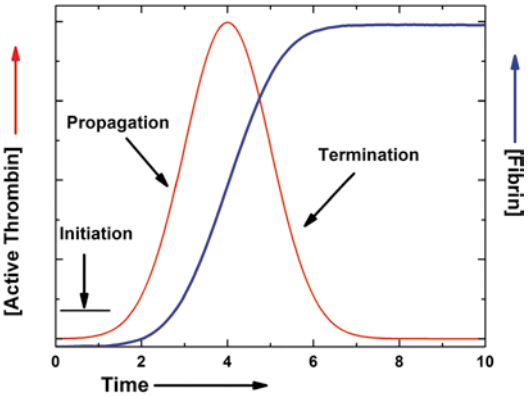


Fig. 6.1 The rate and extent of fibrin formation depends on thrombin. Relationship between thrombin activation (red) and fibrin formation (blue) over time in minutes following initiation of coagulation. In the initiation phase, positive feedback reactions enhance flux through the cascade to yield an explosive increase in thrombin formation in the propagation phase. Termination results following initiation of the anticoagulant reactions and elimination of proteinases by reaction with serpins. Fibrin formation coincides with the burst of thrombin generation

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Additional factor IXa is formed following activation of factor XI by thrombin or via the contact pathway. Factor Xa complexes with factor Va to form the prothrombinase complex, which activates prothrombin to thrombin. Together, these proteolytic reactions drive a coagulation "explosion," dramatically amplifying thrombin generation [2]. The resulting thrombin then cleaves fibrinogen to form an insoluble fibrin clot at the site of vascular injury.

Thrombin also plays a central role in the endogenous anticoagulation and fibrinolytic processes that limit clot extension and prevent pathological venous and arterial thrombosis. Conditions of flow in patent blood vessels – in contrast to the static conditions behind a platelet dam – favor prothrombin cleavage via an alternate pathway to produce meizothrombin. Meizothrombin has anticoagulant properties, binding to thrombomodulin on endothelial cells to catalyze protein C activation and downregulating flux through the cascade by catalyzing the inactivation of factors Va and VIIIa. The clotting process is ultimately shut down by the irreversible inhibition of thrombin and the other serine proteases of coagulation by serine protease inhibitors (SERPINs), such as antithrombin III and alpha-2 antiplasmin, that circulate at high concentrations in plasma and act as suicide substrates [3]. These endogenous anticoagulants prevent spontaneous thrombus formation and balance the extent of hemostasis with the degree of injury. Thrombin also has bidirectional impacts on fibrinolysis, increasing fibrinolysis through inactivation of plasminogen activator inhibitor type 1 and suppressing fibrinolysis through activation of thrombin-activatable fibrinolysis inhibitor [4].

The different reactions comprising the coagulation response and fibrinolysis do not proceed in discrete phases, but, rather, occur contemporaneously. The relative contributions of the procoagulant, anticoagulant, and inhibition reactions on the rate, amplitude, and total amount of thrombin produced determine the resultant fibrin clot formation and subsequent degradation. Because of its key and opposing roles in the regulation of coagulation, it logically follows that physiological perturbations which impact thrombin generation after severe trauma – or during resuscitation – will carry significant risk for dysregulation of the hemostatic response.

Membrane-Dependent Interactions Are Required to Catalyze Thrombin Formation

The prothrombinase complex assembles through reversible protein–protein and protein–membrane interactions on cellular membranes containing phosphatidylserine (Fig. 6.2). A wealth of biochemical and biophysical insights into the assembly and function of prothrombinase have been derived from studies using purified proteins and primarily synthetic phospholipid vesicles containing an optimal ratio of phosphatidylcholine and phosphatidylserine. In vivo, the membrane components important for prothrombinase complex assembly include those provided by activated platelets [5], red blood cells [6], and microparticles [7].

All components – protease, cofactor, and substrate – bind reversibly to membranes containing phosphatidylserine. Factor Xa and prothrombin are vitamin K-dependent proteins. These proteins contain an N-terminal domain called a Gla domain that contains multiple glutamic acid resi-

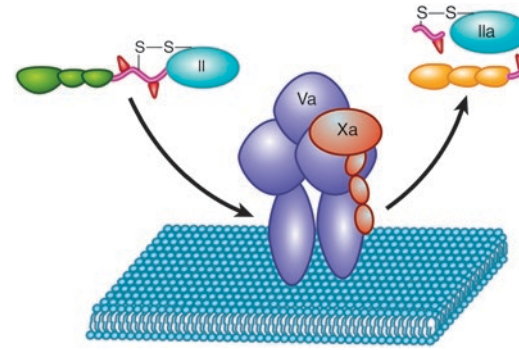


Fig. 6.2 Membrane-assembled prothrombinase. Prothrombinase assembles through membrane-dependent interactions between the cofactor (factor Va) and the protease (factor Xa) on membranes containing phosphatidylserine. The complex cleaves the zymogen prothrombin (II) to active thrombin (IIa) and the activation peptide fragment 1.2 (F12). (Modified from reference [15])

dues that are γ-carboxylated in a vitamin K-dependent reaction. The Gla domain mediates protein binding to negatively charged membranes in a Ca^{2+}-dependent manner [8]. Unlike the vitamin K-dependent proteins, membrane binding by factor Va is not Ca^{2+}-dependent and likely occurs through the two discoidin-like C domains at its C-terminus [8].

Although factor Xa by itself is a competent serine protease with a fully formed active site, alone, it serves as a poor catalyst toward prothrombin. Addition of saturating concentrations of membranes and the cofactor Va dramatically improves the steady-state kinetic constants for prothrombin activation (Table 6.1). These improvements in substrate affinity and catalytic activity of factor Xa, attributed to the effects of membranes and the cofactor factor Va, result in a 500,000-fold increase in the rate of thrombin formation at the physiological concentration of prothrombin.

This phenomenon of cofactor-dependent enzyme activity is generalizable to the entire coagulation cascade, as comparable functional changes accompany the assembly of other coagulation enzyme complexes containing homologous constituents that interact in an analogous way [8]. These functional effects are important, on the one hand, because they promote highly accelerated zymogen activation at the site of vascular damage leading to rapid clot formation. On the other hand, the paradoxically poor activity of the protease alone in the absence of membranes and cofactor limits thrombin formation beyond the site at which damaged or activated cells expose phosphatidylserine. Factors Xa and Va interact with a physiologically irrelevant affinity (Kd ~2 μM) in solution. Enzyme assembly requires the initial and independent binding of factors Xa and Va to the membrane surface followed by their surface-limited interaction to form prothrombinase with ~1000-fold enhanced affinity (Kd ~1 nM) [11, 12]. On the membrane surface provided by activated platelets, factor Va binding is absolutely essential for factor Xa binding and forms at least part of the receptor for factor Xa [13]. Protein–protein and protein–membrane interactions further contribute to enhanced affinity and permit prothrombinase to assemble efficiently at picomolar concentrations, far lower than that predicted from knowledge of the individual binding constants [11].

Because prothrombin can also bind membranes, it follows that similar phenomena must apply to its delivery and cleavage by the membrane-assembled enzyme. However, the contribution of the thrombin–membrane interaction to the rate of prothrombin activation is substantially less, as thrombin formation is only decreased by fourfold for a prothrombin variant lacking γ-carboxylated glutamic acid residues [14].

Table 6.1 Prothrombin activation requires membranes and cofactor. Kinetic constants and relative rates of prothrombin activation by prothrombinase alone or in the presence of saturating concentrations of membranes and the cofactor Va

Substrate		Prothrombin[a]	
Enzyme species[b]	Km (μM)	Vmax/E_T (s^{-1})	Relative rate[c]
Xa	84	0.01	1
Xa/PCPS	0.7	0.05	203
Xa/Va/PCPS	0.4	108	512,000

[a]Kinetic constants for bovine prothrombin cleavage by bovine Xa alone [9], or for the activation of human prothrombin by human enzyme constituents in the presence of membranes [10]

[b]The enzyme species correspond to Xa alone, Xa saturably bound to phosphatidylcholine and phosphatidylserine (PCPS) membranes, and Xa assembled into prothrombinase (Xa/Va/PCPS) using saturating concentrations of membranes and Va

[c]Relative rates at 1.4 μM prothrombin calculated from the steady-state kinetic constants

Prothrombin Activation

The enzymatic mechanisms regulating prothrombinase specificity, the ordered and sequential cleavage of prothrombin, and the steps involved in the transition from zymogen (prothrombin) to protease (thrombin) were elucidated through a series of elegant biochemical experiments by Krishnaswamy and others [15]. Thrombin formation requires proteolysis at two sites within prothrombin. Consequently, there are two possible pathways for prothrombin activation (Fig. 6.3). Initial cleavage of prothrombin following Arg^{271}

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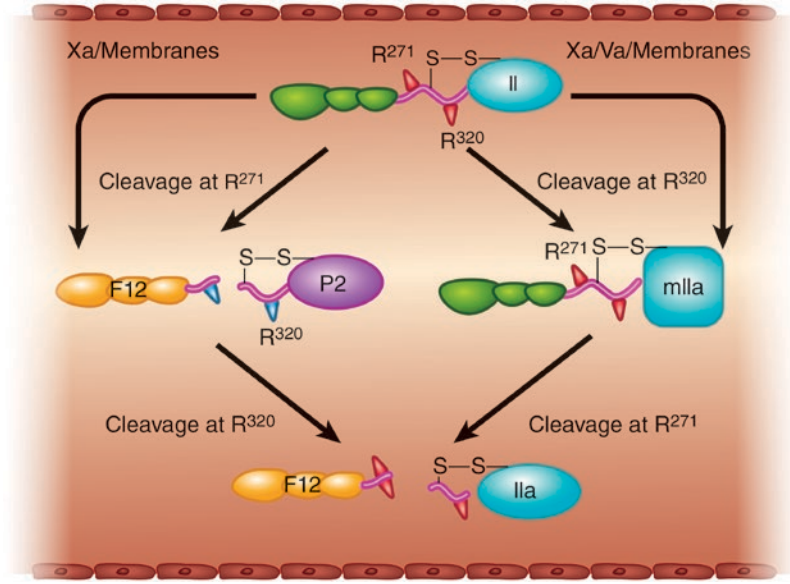


Fig. 6.3 Pathways for prothrombin activation. The conversion of prothrombin to thrombin results from cleavages following Arg^{271} and Arg^{320}. Initial cleavage following Arg^{271} yields the pathway on the *left* and produces the zymogen, prethrombin 2 (P2), and the propiece, fragment 1.2 (F12), as intermediates. P2 requires further processing at Arg^{320} to yield thrombin. The pathway on the *right* arises from initial cleavage following Arg^{320} which produces the protease meizothrombin (mIIa) as an intermediate. Further cleavage following Arg^{271} is required to yield IIa and the propiece, F12. The *left arm* of the pathway predominates in the absence of Va while activation almost exclusively proceeds through the *right arm* for prothrombinase. Xa, factor Xa; Va, factor Va. (Modified from reference [15])

yields the N-terminal pro-piece fragment 1.2 (F12) and the zymogen prethrombin 2 (P2), which is then further cleaved following Arg^{320} to yield the disulfide-linked two-chain form of thrombin. Cleavage of the bonds in the opposite order yields the active protease meizothrombin (mIIa) as an intermediate. Its subsequent processing at Arg^{271} yields the final reaction products.

The cleavage pathway that predominates is dependent on the presence of factor Va and either adequate phosphatidylserine content of membranes or the ability of prothrombin to bind the membrane surface [16]. In the absence of factor Va, the reaction proceeds primarily via cleavage at Arg^{271} leading to the formation of P2 and F12 as intermediates. When prothrombinase is assembled with saturating concentrations of factor Va and membranes with high phosphatidylserine content (25% w/w), such as on activated endothelial cells, red blood cells, and cell-derived microparticles, prothrombin activation proceeds essentially exclusively via initial cleavage at Arg^{320} and the formation of meizothrombin as an intermediate [17].

On the membrane surface of activated platelets, prothrombin cleavage via prothrombinase proceeds via initial cleavage at Arg^{271} to yield the P2 intermediate followed by cleavage at Arg^{320} to produce thrombin [18]. Use of the P2 pathway optimizes the procoagulant activity expressed by activated platelets by limiting the anticoagulant functions of the alternate intermediate, meizothrombin. In further support of this notion is the observation that prothrombinase assembled on activated platelets under flow at typical venous shear rates does not release P2 and is rapidly converted to thrombin [19]. Therefore, on platelets, the conventional pathway prevails and the only active product is activated thrombin. However, phosphatidylserine expression on activated endothelial cells, red blood cells, and cell-derived microparticles can upregulate meizothrombin/thrombomodulin expression leading to localized and/or systemic anticoagulation. As with plate-

lets, factor Xa only binds to red blood cells in the presence of factor Va [6]. However, in contrast to platelets, prothrombin activation proceeds through the meizothrombin intermediate on red blood cells [6]. Similar observations have been made using microparticles derived from washed platelets [20] and isolated from healthy individuals or trauma patients (Bouchard, *unpublished observations*).

The cleavage step from meizothrombin to thrombin is impacted by blood flow. In static systems, such as near a fibrin dam, meizothrombin is rapidly converted to thrombin [16]. However, under flow meizothrombin is a stable product [19]. It can bind membranes with approximately the same affinity as does prothrombin allowing its accumulation on the membrane surface [21]. Meizothrombin not only functions as an anticoagulant by catalyzing protein C activation, but also, it is defective in several of the procoagulant activities of thrombin [22, 23]. Consistent with this is the observation that meizothrombin inhibits thrombosis in a mouse model of acute arterial injury [24]. However, it is interesting to note that thrombin and meizothrombin can activate factor XI to XIa with equal efficiency [25]. In addition, meizothrombin is not inhibited efficiently by heparin–antithrombin and is a potent vasoconstrictor [26].

Thrombin and Traumatic Bleeding

Aberrant thrombin generation is one of the fundamental mechanisms of trauma-induced coagulopathy [27]. Hemodilution, hypothermia, and acidosis contribute to impaired thrombin capacity and coagulopathy, in a "vicious bloody cycle" that promotes hemorrhage [28]. Each of these three factors independently decreases the enzymatic rate of thrombin generation. Hemodilution caused by the infusion of crystalloids during resuscitation decreases the concentration of thrombin and its cofactors. Thrombin activation is temperature dependent and optimal at 37 °C. Hypothermia after trauma, resulting from environmental exposure from skin exposure for examination and hemorrhage control, or in the operating room when the thoracic or abdominal cavities are opened, also impairs thrombin generation. Hypothermia and blood dilution act additively to impair thrombin generation [29, 30]. Similarly, acidosis resulting from anaerobic metabolism during shock can further impair thrombin generation [31, 32].

Trauma surgery practices including transfusion protocols and damage control surgery address iatrogenic causes of coagulopathy and improve the capacity for thrombin generation. Minimizing crystalloid infusion and the early use of plasma will decrease the dilution of thrombin and its cofactors. Transfused red blood cells not only provide increased oxygen carrying capacity, but also promote clot formation by providing membrane surfaces to support meizothrombin and thrombin formation [6, 33]. Massive transfusion protocols guide rapid resuscitation to help prevent or correct metabolic acidosis and coagulopathy. Damage control surgery, staged in multiple operations over several days, can also help prevent hypothermia and the resulting impairment in thrombin generation.

Thrombin and Pathologic Thrombosis After Trauma

The coagulation status of trauma patients may oscillate from hypercoagulable to bleeding coagulopathy and back to hypercoagulable in the hours and days after injury. Severe trauma patients often demonstrate elevated procoagulant activity in their blood, both in the acute phase immediately following injury and also at later time points days or weeks later. This hypercoagulable state predisposes patients to clot formation at a distance remote from the site of initial vascular injury, increasing the risk of systemic pathology including venous or arterial thrombosis and disseminated intravascular coagulation. These complications contribute to morbidity and mortality after trauma.

To some extent hypercoagulability may be explained by elevated expression of tissue factor following tissue damage. Factor XIa has also been shown to be a marker of trauma severity,

and alterations in circulating factor XIa may contribute to TIC [34]. Back-activation of factor XIa by thrombin is greatly stimulated by platelet polyphosphate, promoting further prothrombin activation via activating factor V. Therefore, increased thrombin generation in the context of platelet activation can promote a positive feedback loop with factor XI.

Other contributors to pathologic thrombosis include cellular debris, particularly histones and microparticles [35–37]. Transfusion products contribute to this process; ageing, leukocyte-depleted packed red blood cells generate microparticles over time [33]. Microparticles may influence clot formation via the delivery of coagulation proteins to sites of vascular injury to promote thrombin generation or sequestration of coagulation proteins to dampen the response. Trauma patient blood has an increased concentration of microparticles (approximately fourfold) as compared to healthy controls (Bouchard, *unpublished observations*) that more efficiently support thrombin generation (Fig. 6.4).

Histones increase thrombin generation by impairing thrombomodulin-dependent protein C activation [35]. Histones also promote thrombin generation via platelet-dependent mechanisms, through actions on platelet Toll-like receptor 2 and 4 [38]. Histones also act on endothelial cells through a noncanonical calcium entry pathway that impairs vasodilation and leads to calcium overload and cell death [39]. This can further exacerbate the already hypercoagulable state and contributes to inflammation, microvascular thrombosis, and multiorgan failure. Elevated levels of histone–DNA complexes correlate with adverse clinical outcomes in adult and pediatric trauma patients [40, 41]. Blocking the histone-mediated cascade has the potential to impact clinical conditions including trauma [27].

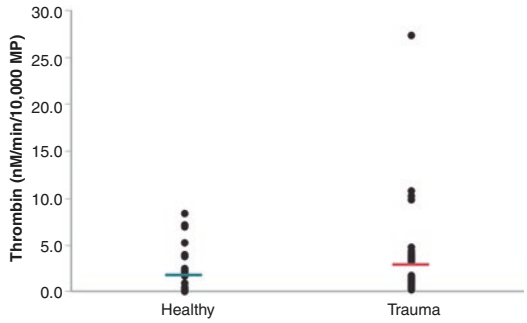


Fig. 6.4 Microparticles from trauma patients support increased rates of thrombin generation. Microparticles were isolated from plasma from healthy individuals or trauma patients as described [33]. Rates of thrombin formation were determined using 10,000 microparticles, 20 nM FVa, 50 pM FXa, and prothrombin (1.4 μM) using a discontinuous assay as described previously [33]. The data are expressed as the rate of thrombin formation over time for 35 healthy controls or patients. The green and red bars represent the mean rate for each population

Vasomotor, Barrier, and Inflammatory Functions of Thrombin

Thrombin has additional vascular effects that are important for tissue repair after injury. Thrombin is a potent vasoconstrictor, recruiting vascular smooth muscle to help achieve hemostasis. In vascular smooth muscle cells, thrombin acts on protease-activated receptors PAR-1, PAR-3, and, to a lesser extent, PAR-4 to induce vasoconstriction and also vascular proliferation, migration, matrix biosynthesis, and production of inflammatory mediators [42]. The alternate prothrombin cleavage product, meizothrombin, has even greater vasoconstrictive activity than thrombin in arteries [26]. Thrombin promotes permeability and leukocyte migration through the endothelial barrier, and the endothelial targets of thrombin signaling, PAR-1, PAR-2, and PAR-4, contribute varying degrees of endothelial Ca^{2+} mobilization via inositol 1,4,5-trisphosphate Ca^{2+}. These changes in Ca^{2+} increase permeability [43].

Future Directions: Monitoring Thrombin Generation During Resuscitation

Direct thrombin generation assays (e.g., calibrated automated thrombogram) have been used to quantify procoagulant activity in several diseases including venous thromboembolism [44, 45] and coronary artery disease [46], as well

as in trauma patients [47]. This technology has the potential to identify real-time hypo- and hypercoagulable states [48]. Thrombin generation does not necessarily correlate with plasma-based assays such as PT or aPTT measurement, and whole blood thrombin generation results are also distinct from plasma thrombin generation in both healthy volunteers and trauma patients [47]. Studies to understand thrombin generation in trauma are ongoing [27]. Point-of-care measurement of thrombin may provide critical information to understand a patient's coagulation status and help guide massive transfusion protocols after trauma.

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Platelets

7

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Introduction and Review of Clot Formation

Platelets are small, anucleate, subcellular-derived fragments from megakaryocytes, and they are key effector cells in hemostasis, vascular homeostasis, and immunoregulation. They provide the structure and functions necessary for assembly of procoagulant proteins that generate large amounts of thrombin, ultimately leading to fibrin polymerization and clot formation. The "cell-based model of hemostasis" was initially described by Hoffman and Monroe and significantly advanced conceptualization of clot formation in the setting of injury [1]. In their model, clot formation is described in three distinct phases: initiation, amplification, and propagation. In the initiation phase, a break in the vessel wall exposes tissue factor leading to binding and activation of clotting factors, including production of small amounts of thrombin. Platelets begin to adhere to the extravascular matrix and exposed subendothelial cells through interactions with tissue factor, von Willebrand factor (vWF), and other proteins including collagen, forming an initial platelet plug. Next, in the amplification phase, platelets become activated in large part by thrombin and through glycoprotein VI receptor interactions with collagen. These steps result in the release and mobilization of large amounts of calcium, which triggers conversion of the platelet structure from discoid to spheric and induces degranulation with subsequent release of multiple procoagulant factors [2, 3]. These factors include adenosine diphosphate, thromboxane A2, serotonin, and factor V. Platelet activation also induces conformational changes leading to glycoprotein IIb/IIIa activation and enabling its ability to bind fibrinogen. The platelet cellular surfaces allow for co-localization of activated clotting factor complexes, resulting in significant production of thrombin in the propagation phase, ultimately leading to fibrin clot formation [1] (see Figs. 7.1 and 7.2).

However, following injury, alterations in platelet biology are increasingly recognized and implicated in the development of TIC. Post-injury thrombocytopenia is associated with bleeding, progression of brain injury, and mortality [5, 6]. In addition, evidence suggests that in the absence of thrombocytopenia, injured patients have impaired platelet biology including increased platelet activation, impaired platelet aggregation, impacts on endothelial and fibrinolytic pathways, and changes in immunoregulation.

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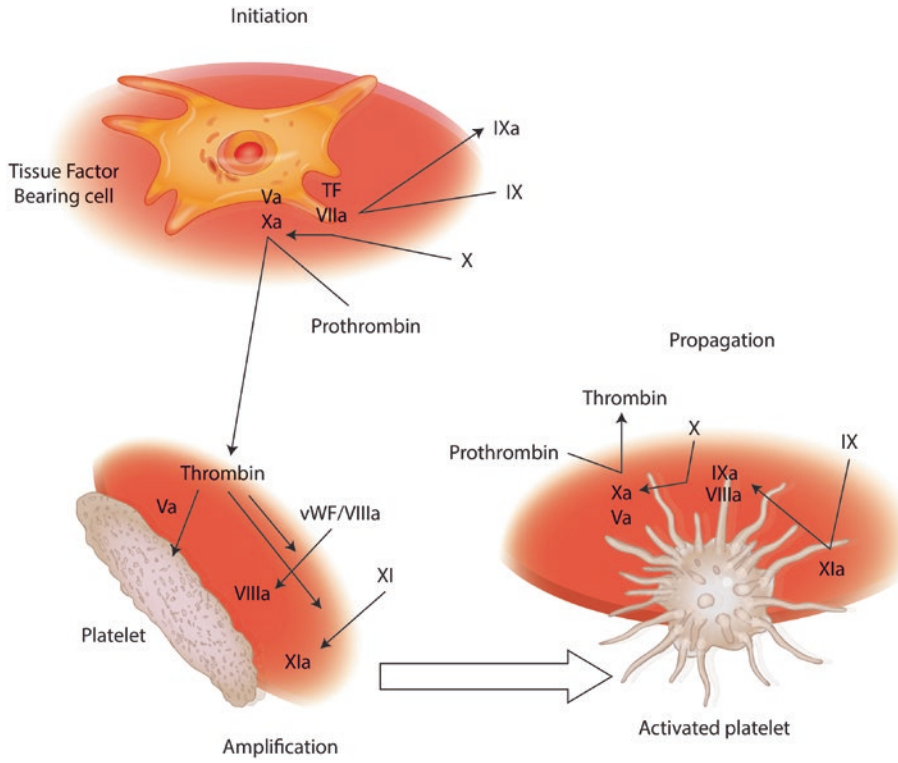


Fig. 7.1 A Cell Based Model of Coagulation. The three phases of coagulation occur on different cell surfaces: Initiation on the tissue factor-bearing cell; Amplification on the platelet as it becomes activated; and Propagation on the activated platelet cell surface. (Modified from Hoffman and Monroe [1])

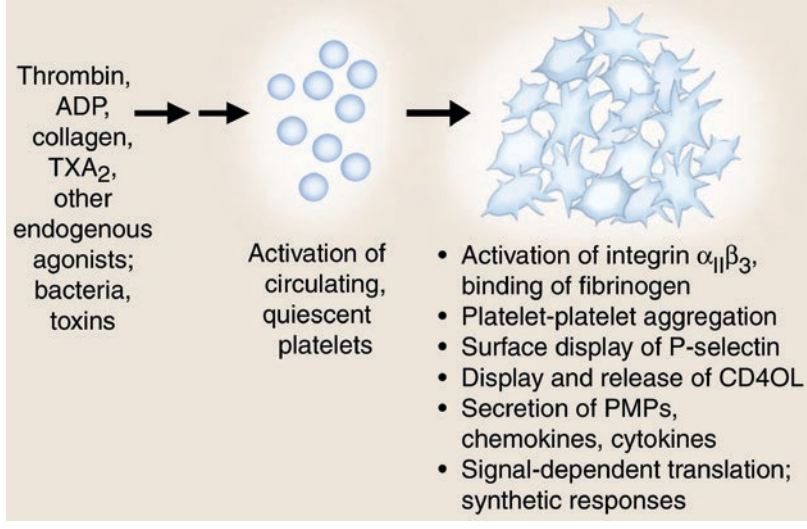


Fig. 7.2 Activation responses of platelets mediate the critical events in hemostasis and inflammation. Platelets activated by agonists recognized by G-protein-coupled receptors, including thrombin, ADP, and TxA_2 or by immunoreceptors such as GPVI, a collagen receptor, deliver outside-in signals to human and murine platelets, resulting in rapid and, in some cases, sustained functional responses that mediate key events in hemostasis and inflammation, and often provide molecular links between hemostatic and immune pathways and responses. Activated platelets also release microvesicles that can initiate or amplify hemostasis and inflammation. (Figure and caption reproduced with permission from Viera-de-Abreu et al. [4])

These functional changes have primarily been studied using ex vivo and in vitro models, including flow cytometry, platelet aggregometry, viscoelastic assays, and more recently, microfluidic studies [5, 7–11]. Our understanding of how these findings correlate to molecular mechanisms and consequent in vivo pathobiology remains incomplete [7–10, 12]. We will highlight key studies in the literature and identify future avenues for investigation where gaps in our knowledge lie.

Platelet Biology in Trauma-Induced Coagulopathy

Thrombocytopenia

Lower platelet counts in trauma patients are associated with worse outcomes including increased bleeding, progression of intracranial hemorrhage, and higher mortality [6, 13]. In fact, in traumatic brain injury (TBI), a platelet count <100,000/μL was shown to have an ninefold adjusted risk of death [13]. The association between platelet count and poor outcomes after injury is not surprising, given it has been shown that platelets contribute significantly more to clot strength than fibrinogen, as measured by thromboelastography [14]. While decreases in platelet number do occur after trauma, which may be multifactorial including secondary to dilutional effects of resuscitation as well as consumptive processes, the majority of patients present with normal platelet counts immediately following injury [12] and the primary platelet phenotype associated with TIC appears to be *qualitative* in nature rather than *quantitative* [7, 8, 10]. Specifically, in two prospective studies evaluating platelet activation and aggregation in severely injured patients, mean initial platelet counts were well within normal ranges (226,000/μL and 275,000/μL) [7, 8], yet 45% of these patients had evidence of impaired platelet aggregation on admission [8]. As is typical, it was not until 24–48 h of admission that mean platelet counts nadired below 150,000/μL [7, 8].

Increased Platelet Activation and Impaired Platelet Aggregation

Nearly half of trauma patients who present with normal platelet counts have evidence of impaired platelet aggregation [8]. Multiple authors have found significant associations between this impaired platelet aggregation and mortality [8, 10]. In 2001, Jacoby et al. analyzed platelet activation in 100 trauma patients compared to 10 healthy controls and showed increased activation but impaired aggregation in the trauma group [7]. Platelet activation was measured through biomarker analysis of translocation of P-selectin, glycoprotein IIb/IIIa, and platelet microparticles. Platelet aggregation was assessed using a platelet function analyzer (PFA-100), which measures the time required for shear-induced occlusion of porous membranes containing platelet agonists (ADP, collagen, and epinephrine) which induce clot formation (see Table 7.1 for a description of commonly used assays of platelet function in trauma). They identified significantly increased platelet activation across all trauma patients compared to controls, including increased platelet microparticles in non-survivors and in patients with TBI [7]. In addition, this study was notable in that it identified *decreased* closure times using the PFA-100 on admission compared to controls overall (suggesting enhanced platelet-driven clot formation); however, closure times were markedly *prolonged* among non-survivors at all time points indicative of impaired clot formation in this group. It is hypothesized that endothelial damage and the release of tissue factor may play a role in platelet activation and impaired platelet aggregation (a functional "platelet exhaustion") that is identified in these studies and that is associated with worse outcomes, increased transfusion requirements, and mortality [7, 14].

In 2012, Kutcher et al. published findings describing impaired platelet aggregation in trauma patients using multiple electrode impedance aggregometry (Multiplate®) [8]. The Multiplate® device measures platelet aggregation by electrical impedance in response to a variety of agonists in samples of whole blood

Table 7.1 Commonly used assays and technologies for assessing platelet function. (Table adapted with permission from Lordkipanidzé [15])

Tests of platelet function in trauma				
Name of test	Methodology	Advantages	Limitations	Applications/uses
Tests with clinical and research applications				
Light transmission aggregometry (LTA)	Measures changes in light transmission in platelet-rich plasma in response to a variety of agonists to determine platelet aggregation	Considered gold standard for aggregometry, widespread use	Time consuming, significant sample preparation and volume required, lacks incorporation of other circulating blood cells, endothelium, and flow	Identification of platelet aggregation defects Monitoring of antiplatelet therapy Research
Lumi-aggregometry	Combines LTA or whole blood aggregometry (WBA) with measurement of nucleotide release as a marker of degranulation	Increased sensitivity by measuring both degranulation and aggregometry	No common standard reference ranges, lacks incorporation of endothelium and flow	Identification of acquired and inherited platelet storage and release disorders
Platelet function analyzer (PFA-100®, PFA-200®)	Measures time to shear-induced occlusion of microporous membrane containing agonists using whole blood	Incorporates flow and shear stress effects Point-of-care testing	Dependent upon platelet count and hematocrit, lacks incorporation of endothelium	Screening test for platelet function defects Research
Plateletworks®	Utilizes changes in platelet count pre and post activation to determine aggregation in whole blood	Point of care, small volume of blood required	Indirect test, lacks incorporation of endothelium and flow	Used in cardiology, surgery for monitoring of antiplatelet drugs
Whole blood aggregometry (WBA) (Multiplate®, VerifyNow®)	Measures changes in electrical impedance in response to a variety of agonists to determine platelet aggregation in whole blood	Reproducible Variety of agonists can be tested	Labor intensive, missing endothelium and flow	Determine platelet aggregation defects Monitoring antiplatelet therapy VerifyNow®: automated point of care WBA Research
Tests primarily used for research				
Calcium mobilization assays[a]	Measures calcium mobilization in response to variety of agonists in platelet-rich plasma	Provides global measure of platelet activation	Not in widespread use, lacks incorporation of other circulating blood cells	Research
Flow cytometry	Measures markers of platelet activation in plasma or whole blood using fluorescently labeled antibodies	Quantifies a variety of surface markers and cellular interactions specific to platelet activation	Costly, labor intensive, lacks incorporation of endothelium and flow	Research

Table 7.1 (continued)

Tests of platelet function in trauma				
Name of test	Methodology	Advantages	Limitations	Applications/uses
Microfluidic technologies	Miniaturized multichannel devices which measure aggregation and other measures of platelet function using whole blood	Ability to modulate flow and sheer stress Customizable to create multiple conditions	Lacks incorporation of endothelium and signaling	Research
Platelet mapping (TEG®-PM™)	Traditional thromboelastography (TEG) combined with platelet agonists, uses whole blood sample	Also provides overall assessment of hemostasis	Limited agonist activation, labor intensive, lacks incorporation of endothelium and flow	Research Point-of-care testing

[a]See Verni et al. [5] and Lee and Diamond [16] for further reference

(Table 7.1). In this study, they measured platelet aggregation in 101 trauma patients and found evidence of impaired platelet aggregation in response to at least one of four agonists in 45% of their pre-resuscitation samples and in 90% of patients at some point in their hospitalization [8]. Platelet "hypofunction" on admission, defined as less than the fifth percentile of reference range for the Multiplate® platform, was associated with a tenfold increased risk of mortality, lower Glasgow Coma Scales, and higher mechanical ventilation requirements, independent of platelet counts [8]. Out of the four agonists they tested (adenosine diphosphate [ADP], arachidonic acid [AA], thrombin receptor–activating peptide [TRAP], and collagen), impaired responses to AA and collagen were predictive of mortality [8]. Similarly, Soloman et al. undertook a similarly sized study using impedance aggregometry to evaluate platelet aggregation in response to ADP, TRAP, and collagen in 163 trauma patients [10]. The incidence of impaired platelet aggregation was overall lower, but this may be attributable to the fact that their population had lower injury severity scores. They specifically found that impaired responsiveness to ADP and TRAP was associated with increased mortality [8, 10].

Microfluidic technologies allow for development of ex vivo models that may more closely simulate in vivo clot formation by nature of their ability to assess platelet activation and aggregation and other parameters of clot formation under a range of flow and shear stresses (replicating arterial or venous pressures; Table 7.1). Microfluidic devices have been used to measure coagulation status in hemophiliacs and can evaluate the effects of antiplatelet medications [17]. These systems can also allow for assessment of fibrin polymerization and fibrinolysis, thrombin production, and clot stability, and fluorescent antibodies can be used to allow for differential monitoring of platelets, fibrin, and thrombin [18–20]. This technology is now being applied to evaluate platelet impairments in the context of trauma. For example, in a study of 20 trauma patients by Li, Diamond, and colleagues, the investigators identified reduced platelet aggregation and collagen response in the trauma group compared to controls using microfluidic systems [9]. Their data suggested two phenotypes of impaired platelet function: some patients had impaired aggregation suggestive of dysfunctional glycoprotein VI, while others showed impaired ability to develop clot after the initial layer of aggregating platelets formed, suggestive of decreased ADP or TXA2-mediated clot growth [9]. They also noted the important role that red blood cells play as they promote margination of platelets to vessel walls and release ADP, which further induces platelet aggregation. Interestingly, in this study, there was no correlation between impaired platelet aggregation and injury severity

scores or TBI. Other studies have utilized microfluidic systems to delineate mechanisms responsible for clot stability under shear stress [21] and to develop more sensitive models of vessel injury [18]. However, to date the microfluidic platforms available primarily do not incorporate endothelial contributions to platelet behavior, but rather focus on the effects of platelet activation and shear stress. The absence of the bidirectional platelet-endothelial signaling in these models remains a major limitation of this technology and the majority of other assays of platelet behavior (Table 7.1).

Platelet-Regulated Endothelial Integrity

Platelets also have a bidirectional and reciprocal relationship with endothelium to promote vascular homeostasis, and this may have important implications for TIC [22]. Platelets regulate and maintain the endothelium through release of several factors. These include sphingosine-1, which stabilizes endothelial cell tight junctions and inhibits apoptosis, stromal cell–derived factor-1, and others which promote angiogenesis and endothelial cell maturation [22]. Conversely, endothelial cells also play an important role in regulation of platelets, as they promote platelet production from megakaryocytes and release vWF which mediates platelet aggregation (see Table 7.2). This relationship is notable because injured patients with the most robust increase in platelet activation and impairment in platelet aggregation include those with injuries characterized by significant tissue and endothelial damage, including TBI, higher injury severity scores, shock, and death [7, 8].

In a recent study by Kornblith et al., the authors hypothesized that the excessive release of vWF from damaged endothelium may provide the mechanistic explanation for impaired platelet aggregation seen in TBI, since the blood-brain barrier is rich in endothelial cells [23]. In a cohort of 233 patients, they found impaired platelet aggregation in platelet aggregometry in response to ristocetin stimulation (which induces platelet agglutination mediated by vWF) in patients with TBI compared to controls [23]. Ristocetin-dependent platelet agglutination is dependent upon platelet interactions with vWF. These findings were therefore suggestive of a qualitative or quantitative deficit in the vWF axis mediating impaired platelet aggregation after TBI, which was also supported by an observed increase in circulating factor VIII activity (potentially due to impairments in qualitative or quantitative carrying capacity of vWF). These findings are significant in that they begin to identify alternative mechanisms of impaired measures of platelet aggregation and therefore potential therapeutic targets outside platelet transfusion. Further investigations are required to better understand the relationships between platelets and the endothelium in the setting of injury.

Table 7.2 Summary of platelet-endothelial interactions. See Nachman and Rafii (2008) for further reading [22]

Key signaling between platelets and endothelial cells	
Factors produced by megakaryocytes or released by platelets	*Impact on endothelial integrity*
Sphingosine-1	Stabilizes endothelial tight junctions, inhibits apoptosis
Vascular endothelial growth factor A Stromal cell–derived factor 1 Angiopoietin 1 Epidermal growth factor Brain-derived neurotrophic factor Platelet-activating factor vWF	Proangiogenic cytokines and trophogens supporting endothelial cell growth Stabilization of tight junctions Contribute to revascularization, tumor growth, plaque progression [15]
Factors produced by endothelial cells	**Impact on platelet function**
Stromal cell–derived factor-1 Thrombopoietin Stem cell factor c-kit ligand Fibroblast growth factor-4	Megakaryocyte trophogens
vWF	Platelet adhesion and aggregation

Platelet-Regulated Fibrinolysis

Abnormal fibrinolysis is a central feature of TIC that has recently been characterized in large patient cohorts [24, 25]. Both excessive fibrinolysis (hyperfibrinolysis) and impaired fibrinolysis (fibrinolysis shutdown) are present in critically injured patients and have significant associated morbidity and mortality [24, 25]. Furthermore, research suggests platelets are important regulators of fibrinolysis [26–29]. For example, in vitro studies demonstrated that platelet cell lysate effectively inhibits fibrinolysis [26]. The proposed mechanism is platelet release of alpha antiplasmin-2 and plasminogen activator inhibitor-1, which both inhibit fibrinolysis [27, 28]. Moore et al. hypothesized that in trauma patients, defects in platelet responsiveness to agonism might mediate increased sensitivity to fibrinolysis [29]. In a cohort of severely injured patients, the authors identified that although there were stronger clots measured by thromboelastography compared to uninjured controls, these clots were more sensitive to tissue plasminogen activator (tPA)-mediated fibrinolysis, which correlated to platelet inhibition measured by impaired response to ADP agonism [29]. At the cellular level, platelet-platelet interactions lead to formation of a dense core of prothrombotic and antifibrinolytic platelets within a thrombus, while a protective shell of ADP-activated platelets forms the exterior [30, 31]. Some degree of inhibition to ADP might be adaptive in the setting of trauma in order to prevent clot formation in uninjured vascular beds; however, excessive platelet inhibition to ADP may contribute to a hyperfibrinolysis phenotype, which is clearly maladaptive and associated with worse outcomes and mortality after trauma. Additional research is needed to further delineate the relationship between platelets and fibrinolysis, the associations with outcomes including thromboembolic complications, and potential therapeutic interventions.

Platelet-Regulated Immunoactivation

As our understanding of post-injury platelet biology and the multifaceted roles platelets have in TIC continue to evolve, platelets are now understood to be not only central mediators of hemostasis, but also important signaling and effector cells in the immune and inflammatory response to injury. Abnormalities and overactivation of these immune and inflammatory properties of platelets have been implicated in thrombotic events, inflammation, and organ failure [32–36].

In the setting of injury, platelet activation has been found to lead to interactions with leukocytes, which was associated with impaired platelet aggregation and decreased clot strength [37]. Platelets express receptors and release mediators that facilitate migration of leukocytes to sites of injury and drive expression of inflammatory cytokines [38]. For example, thrombin and platelet thrombin receptor (PAR4) mediate leukocyte recruitment to the platelet plug, and P-selectin allows leukocyte interaction with thrombus [39]. The interface between hemostasis and the immune system facilitated by platelets may be beneficial in terms of directing immune cells to sites of injury where microbial contamination may have occurred (see Fig. 7.3). However, these processes are also thought to be maladaptive, particularly in the setting of shock. Toll-like receptor 4 (TLR4) is an important receptor expressed on platelets which has been studied specifically in the setting of hemorrhagic shock [33, 34]. In a mouse model, TLR4 knockout mice were protected from shock and the resuscitation-induced inflammatory response and endothelial dysfunction [34]. Toll-like receptors have been proposed as a potential therapeutic target to prevent the organ dysfunction associated with resuscitation following hemorrhagic shock [32].

Although much of the focus in the study of TIC has been on states of hypocoagulability, there is strong evidence that changes in platelet biology and platelet-immune cell interactions may contribute to hypercoagulability and venous thromboembolism, which remains an important cause of morbidity and mortality after injury [35, 40–43]. The interaction of platelets with neutrophils and monocytes leads to a pro-inflammatory and procoagulant phenotype. Binding of platelets to neutrophils and monocytes results in the release of multiple inflammatory cytokines, platelet mic-

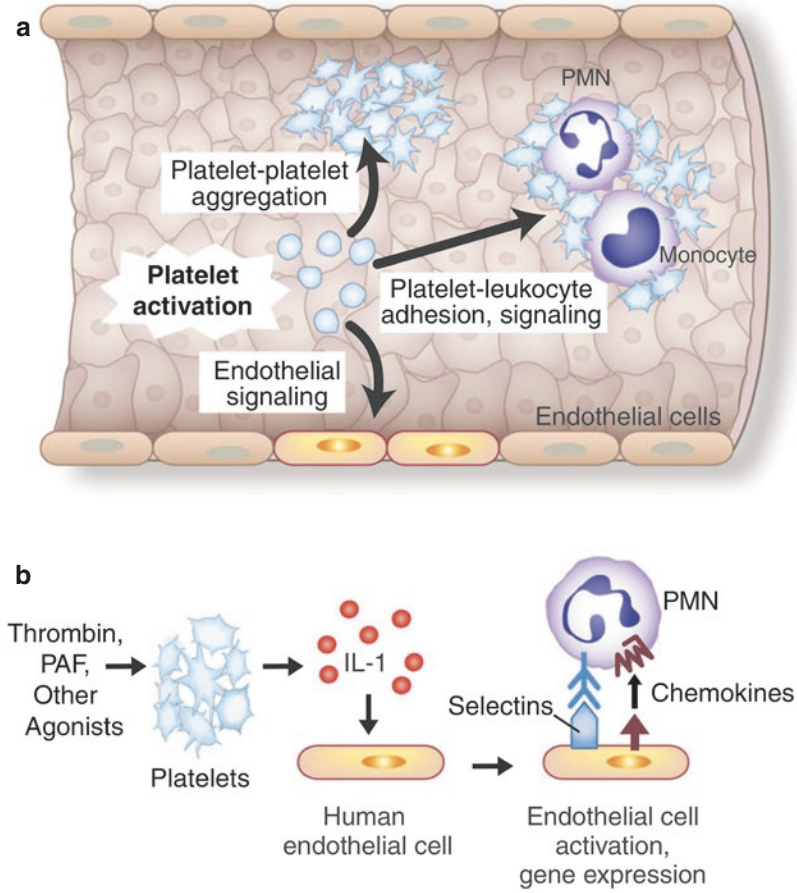


Fig. 7.3 Adhesive and signaling mechanisms of activated platelets mediate critical cell–cell interactions in hemostasis, inflammation, and immune responses. (**a**) The activation of platelets mediates platelet–platelet aggregation, platelet–leukocyte adhesion and intercellular signaling, and interactions with inflamed and injured endothelial cells. Endothelial cells release factors that inhibit or modify these interactions, including prostacyclin and nitric oxide, under basal and stimulated conditions. […] (**b**) The release of IL-1 by activated platelets mediates platelet–endothelial interactions. The activated human and murine platelets release IL-1 in solution and associated with microvesicles. […] IL-1β released in microvesicles from activated platelets also induces human endothelial cells to bind PMNs. This is consistent with many observations demonstrating that IL-1 is a prototypic agonist for "type II" activation of endothelial cells, which includes the synthesis and surface expression of E-selectin, IL-8, and other chemokines, leading to the adhesion and activation of PMNs and monocytes. IL-1 also induces the synthesis of tissue factor dependent procoagulant activity by human endothelial cells. Thus, IL-1-mediated platelet endothelial interactions have the potential to induce and drive key innate and adaptive immune responses and to link inflammation and hemostasis. (Figure and caption reproduced with permission from Rondina et al. [40] and Vieira-de-Abreu et al. [4])

roparticles, and also increased complement and TNF-alpha release [38, 44]. Platelet activation also results in the substantial release of platelet extracellular vesicles, which have been shown to be prothrombotic and contribute to thrombus burden in a murine model of deep vein thrombosis [40]. Direct platelet interactions with monocytes and release of platelet factor-4 (CXCL4) result in increased expression of tissue factor and increased levels of both fibrinogen and factor Xa (see Fig. 7.4) [44]. Furthermore, platelet monocyte aggregation is associated with venous thromboembolism in surgical patients [43]. The role of platelet-leukocyte interactions in trauma patients merits additional investigation. The CD40 ligand is an important antigen expressed on platelets that

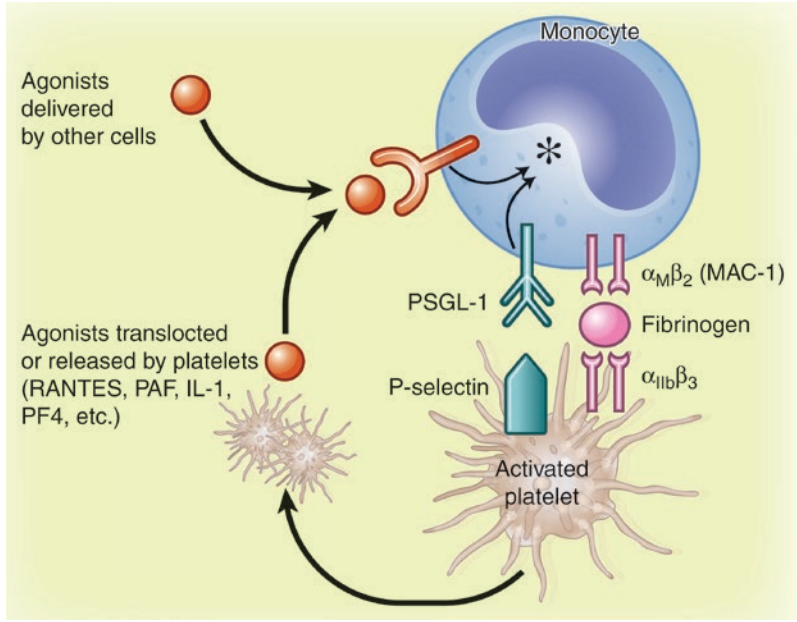


Fig. 7.4 Activated human platelets adhere to monocytes and deliver outside-in signals that induce altered functions. […] Binding of P-selectin to P-selectin glycoprotein ligand-1 (PSGL-1) basally present on the monocyte plasma membrane is critical for this interaction. […] This engages members of the integrin family on monocytes, potentially amplifying the adhesion provided by P-selectin/PSGL-1 and contributing to other adhesive interactions with endothelium, subendothelial matrix, or other blood cells. Outside-in signals (RANTES, PF4, IL-1, PAF, CD40L, etc.) can act in concert with signals delivered by the engagement of PSGL-1 as can agonists delivered by other cell types. (Modified from Viera-de-Abreu et al. [4])

also appears to play an important role in the pro-inflammatory and procoagulant response driven by injury [45]. In a prospective study of 80 trauma patients, Johansson et al. found that higher levels of circulating CD40 ligand were associated with endothelial injury, shock, coagulopathy, and mortality [45].

In addition, injury leads to release of several damage-associated molecular pattern molecules (DAMPS). High-mobility group box 1 (HMGB1) is a DAMP that has been studied in murine models of trauma and hemorrhagic shock [35]. In a study by Vogel, Neal, and colleagues, transgenic mice lacking platelet-derived HMGB1 in a knockout model had increased bleeding and decreased clot formation, organ damage, and platelet aggregation, suggesting a key role for HMGB1 in the development of thrombotic events [35]. HMBG1 appears to promote thrombosis through formation of prothrombotic, neutrophil extracellular traps (NETS) [41]. In a murine model of deep vein thrombosis (DVT), platelet-derived HMGB1 led to increased NET formation, ultimately promoting the development of DVT [42]. The authors showed that platelets deposit HMGB1 on endothelial cells and concluded that this plays a key role in DVT development through monocyte recruitment and tissue factor production, NET formation, and enhanced platelet aggregation [42]. They also showed that inhibition of HMGB1 decreased the extent of thrombus in their murine model, suggesting HMGB1 as a possible therapeutic target [42]. Continuing to advance our understanding of these complex interactions between platelets and immune cells will allow for the development of new strategies that may prevent and mitigate micro- and macrovascular thrombosis and organ failure in trauma patients.

Limitations of Measuring Altered Platelet Biology in Trauma-Induced Coagulopathy

As multiple phenotypes of post-injury platelet biology ranging from physiologic to maladaptive are identified, it has become even more critical to recognize the limitations of measuring platelet biology in vitro. Current point-of-care platelet assays for use in trauma are largely restricted to assays intended for detection of pre-injury platelet inhibition by medications such as aspirin or clopidogrel (Table 7.1) [2, 3]; however, point-of-care testing to determine endogenous platelet impairments remains largely unexplored. A lack of correlation between viscoelastic assays and point-of-care platelet technologies in coagulopathic trauma patients calls to attention the discrepancies present in current testing and the need for diagnostic testing validated to assess platelet biology in the injured patient [11, 46].

Specifically, the clinical significance of impaired platelet aggregation measured using ex vivo and in vitro models has its limitations and controversies (Table 7.1). Like other agonist-based aggregometry assays, Multiplate® was designed to test the effect of antiplatelet therapies. Injury has been shown to endogenously activate platelets, and therefore one could hypothesize that in the setting of endogenous activation, a lack of further response to agonists in vitro may be expected and does not necessarily imply platelet "dysfunction" or "hypofunction" [46]. Impaired platelet aggregation has also been demonstrated in even mildly injured cohorts [11], further questioning whether this finding is truly clinically significant or rather simply a marker of injury. Sirajuddin et al. studied platelet aggregometry in a minimally injured group of 459 trauma patients with median injury severity score of only 5 and found impaired aggregation in response to ADP and AA stimulation, albeit less than in studies of more severely injured patients, but still significantly greater than uninjured controls [11]. Additionally, assays such as platelet function analyzer (PFA-100) have other important drawbacks as PFA-100 is reliant on both hematocrit and platelet count, which can be initially abnormal and often decrease over time after injury [7].

These and other potential limitations make it difficult to interpret the role of impaired platelet aggregation as a true driver of TIC. One of the proposed mechanisms involves the concept of "platelet exhaustion" in which platelets are initially activated on a large scale by injury and subsequently are refractory to further stimulation [7]. However, another possibility is that platelet assays may selectively sample impaired or degranulated circulating platelets, which may be inherently different than the phenotypes of platelets that are incorporated into clots at local sites of injury [12]. In a mouse model of penetrating injury, Stalker et al. described that in the platelet plug there existed a "core region" of platelets exposed to high levels of thrombin and collagen, while the "outer shell" region of platelets was loosely packed and growth depended upon ADP interactions, and therefore some degree of inhibition to ADP could be normal [30].

Studies are ongoing to enhance our understanding and ability to assess post-injury platelet biology, including high-throughput microfluidics measurements (incorporating flow and endothelial environments in a rapid assay), microscopy (light, fluorescence, and electron), mitochondrial respiration, and broadened biomarkers of platelet activation, aggregation, and endothelial and immune interactions [23, 26, 29, 30, 34]. These studies will be critical to ultimately improving and expanding our understanding of post-injury platelet biology and therefore optimal platelet-based treatments for TIC.

Platelet Transfusions in Trauma-Induced Coagulopathy

Finally, the impact of platelet transfusions on outcomes in trauma patients is an important area of active investigation. Given the evidence of impaired platelet aggregation post-injury that is associated with bleeding and mortality outcomes, platelet transfusions would seem an obvious choice of therapy. Indeed, retrospective studies demonstrated associations between higher plate-

let to red blood cell transfusion ratios and improved survival [47, 48], and in the landmark "Pragmatic, Randomized Optimal Platelet and Plasma Ratios" (PROPPR) study, patients randomized to 1:1:1 plasma to platelet to red blood cell to plasma transfusion ratio had a lower risk of exsanguination and improved rates of hemostasis, but no differences in survival [49]. A subsequent secondary analysis of the PROPPR study data did demonstrate that early transfusion of platelets was associated with improvements in both 24-h and 30-day mortality, as well as fewer deaths from exsanguination [50]. This analysis was limited to patients who received only the first cooler of blood products, which in the intervention arm included transfusion of platelets first (followed by red blood cells and plasma in a 1:1:1 ratio), whereas in the control group the first cooler included only red blood cells and plasma [50]. Therefore, this does suggest a beneficial role for early transfusion of platelets for hemostasis.

However, despite evidence supporting the role of platelet transfusion in hemostasis and overall survival, the ability of platelet transfusions to correct post-injury platelet impairments remains uncertain. In a prospective study of trauma patients receiving four or more red blood cell transfusions, platelet aggregation, thromboelastography, and clotting factors were assessed at multiple time points to determine the impact of platelet transfusions on platelet aggregation and fibrinolysis [51]. Platelet transfusions had no apparent impact on restoring platelet aggregation (or platelet count, interestingly), but did increase plasminogen activator inhitibor-1 (PAI-1) levels, decrease tPA levels, and attenuate fibrinolysis rates [51]. While the aforementioned study found no in vitro effect on platelet aggregation, other data suggests that platelet transfusions may actually *worsen* in vitro measures of platelet aggregation, which some have hypothesized to be due to the effect of prolonged storage time [52]. In fact, the impact of platelet transfusions may be more nuanced and time dependent. Recent data suggests that early platelet transfusions after trauma have minimal to negative impacts on platelet aggregation, while transfusions at later time points from injury improve platelet aggregation [53]. One explanation for the lack of improvement in in vitro platelet aggregation following platelet transfusion may be that circulating factors in the plasma of trauma patients inhibit circulating platelets. In a study by Verni et al., healthy human platelets were analyzed in the presence of plasma from trauma patients and were found to have 50% greater inhibition of response to traditional agonists compared to healthy platelets in control plasma [5]. Based on prior work by the same authors, they hypothesized that increased levels of soluble fibrin and fibrin degradation products in trauma plasma may in part be responsible for inhibition of platelet aggregation, through blocking of glycoprotein VI signaling [54].

Beyond data to suggest that platelet transfusions do not improve platelet aggregation after injury and may actually inhibit it are concerns regarding the consequences of platelet transfusion and difficulties defining the optimal platelet transfusion threshold in trauma patients. Some have proposed liberal transfusions to a threshold of >100,000/μL [6]. However, this may have unintended negative impacts, including inflammatory reactions, infectious complications, organ damage, and cost without clear benefit [55]. In a comprehensive review of the literature of transfusion practices in critically ill and in surgical patients, Etchill, Neal, and colleagues demonstrated that transfusion practices are highly variable, transfusion thresholds are based primarily on expert opinion and consensus, and despite the clear associations between thrombocytopenia and poor clinical outcomes, the benefit of platelet transfusions remains unclear [55]. In fact, in patients with hemorrhagic stroke, empiric transfusion of platelets was associated with *worse* outcomes, including increased mortality [56], and platelet transfusions do not improve platelet aggregation or platelet count in the setting of massive transfusion [51]. Finally, studies have demonstrated that early platelet transfusion after injury is strongly and independently associated with organ failure, specifically lung injury [57–59].

Some investigators have proposed using thromboelastography with platelet mapping (TEG-PM)

as a tool that could be used to assess the need for platelet transfusion (rather than platelet count). TEG-PM measures inhibition to ADP agonism and was developed to assess antiplatelet therapy in cardiac patients. A recent study investigated whether the use of TEG-PM would be useful in predicting requirements for platelet transfusions, massive transfusion, or mortality [60]. The authors did find significant platelet inhibition in their trauma population by TEG-PM which was associated with mortality, massive transfusions, and platelet transfusions; however, TEG-PM was not better at predicting outcomes than platelet counts, standard rotational thromboelastography, or clinical markers of injury severity and coagulopathy [60]. While we still do not know the optimal platelet transfusion thresholds in trauma patients (and there may not be one size fits all), we do know that "goal-directed" transfusion practices with thromboelastography have been associated with improved outcomes and decreased platelet and overall transfusions in patients being resuscitated with massive transfusion protocols [61].

Conclusions and Future Directions

Our understanding of the multifaceted roles platelets play in the response to injury has significantly advanced over the past several decades. Increased platelet activation and impaired aggregation have been studied in multiple models and found to be associated with worse outcomes in trauma patients. The next steps will lie in understanding how these findings relate to post-injury platelet biology in vivo and the underlying mechanisms involved, so that improved and targeted therapeutic strategies can be developed. It will also be important to continue to develop an increased understanding of interactions of platelets with the fibrinolysis, endothelial, and immune-mediated pathways and how these relate to outcomes after injury. In addition to hemostatic functions, the immunomodulatory and inflammatory functions of platelets are now being increasingly studied in the context of TIC.

In summary, although alterations in post-injury platelet biology are a consistent finding in trauma patients and have been shown to be associated with worse outcomes and mortality, the biologic mechanisms responsible for these findings remain unsolved. A key unanswered question is to what extent and in what contexts post-injury changes in platelet biology represent adaptive or maladaptive responses. Further research defining the underlying mechanisms are active areas of ongoing investigation and will surely include biomarker studies, microfluidics, platelet mitochondrial health, and microscopy. Ultimately, improving our understanding of the drivers of altered post-injury platelet biology that contribute to TIC will lead to improved therapeutic strategies and interventions for critically injured patients including potential novel synthetic therapies [62], purified platelet-derived products [63], and non-platelet-derived mediators of platelet behavior [23].

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Fibrinogen

8

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Introduction

Fibrinogen is an essential protein for hemostasis and circulates at the highest concentration of all the coagulation proteins. It is also among the most ancient vertebrate hemostatic proteins [1, 2]. In humans, fibrinogen contributes both hemostatic and immunological functions. This chapter focuses on its role as a hemostatic protein where it serves as the substrate for fibrin clot formation, binds platelets, and provides a template for the binding of thrombin as well as proteins of the fibrinolytic system. This chapter will also highlight the clinical aspects of fibrinogen's structure and functions, as they relate to the pathophysiology of trauma-induced coagulopathy (TIC). A standardized nomenclature regarding fibrinogen structure proposed by the International Society on Thrombosis and Haemostasis (ISTH) [3] will be used throughout this chapter.

Structure

Fibrinogen is a 340-kDa dimeric glycoprotein composed of two subunits made up of three polypeptide chains (A-alpha, B-beta, gamma) that are encoded by genes on chromosome 4 (*FGA, FGB, FGG*). These subunits are linked centrally by disulfide bonds in what is known as the central "disulfide knot" contributing 3 overall structural domains [1]. The central "E-domain" contains the N-termini of each polypeptide chain which is flanked by two globular peripheral "D-domains" [4, 5]. The D-domains contain nodular beta and gamma chain C-terminal segments along with a relatively unordered alpha-C terminal chain segment that loops back to the central E-domain (alpha-C domain) [6]. Together, the two alpha-C regions comprise 25% of the total mass of fibrinogen. This configuration gives fibrinogen a trinodular structure that is 45 nm in length and contains 29 disulfide bonds, linking a total of six polypeptide chains (Fig. 8.1). This structure supports fibrin polymerization and serves as a surface, containing motifs for binding of other proteins.

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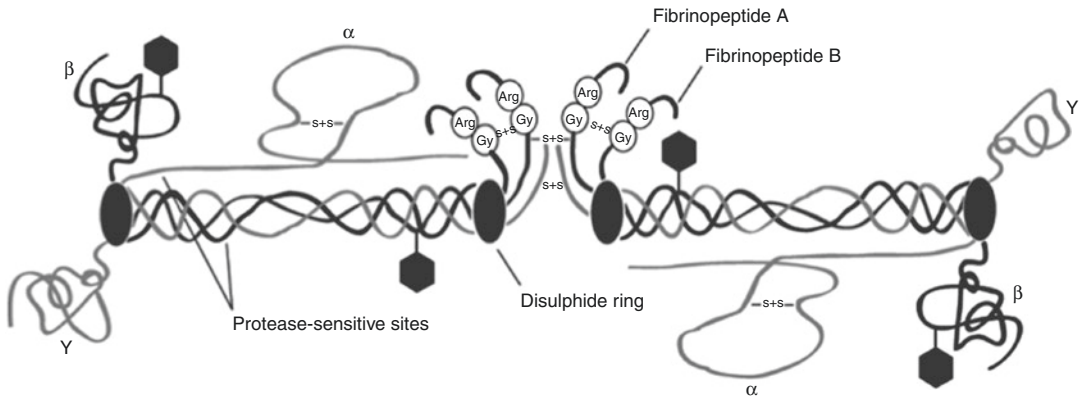


Fig. 8.1 Dimeric structure of fibrinogen formed by homologous alpha (A-alpha), beta (B-beta), and gamma chains. Hexagons represent carbohydrates attached to the protein

Each fibrinogen domain and polypeptide chain contain important elements related to its conversion to fibrin and subsequent coagulation. The E-domain contains ligand binding sites for thrombin where small fibrinopeptides "A" and "B" are liberated, transforming fibrinogen into a soluble fibrin monomer. The gamma chain participates in binding of thrombin [7], platelets via the C-terminal AGDV-containing dodecapeptide (γC-12) [8], factor XIII [9, 10], and fibrinolytic enzymes. The beta chain contains complementary "knob" and "hole" binding sites that contribute to fibrin polymerization and fibrin clot structure [11]. The terminal alpha-C domain contains beta hairpin structures that contribute to fibrin polymerization through lateral aggregation of fibrin protofibrils in addition to multiple platelet and fibrinolytic enzyme binding motifs [8, 12].

The presence of carbohydrates on fibrinogen has important functional consequences. Carbohydrates have been found on B-beta and gamma chains, while the A-alpha chain was reported to be devoid of carbohydrates [13]. An example comes from patients with cirrhosis who have hypersialated fibrinogen that forms clots with thin fibers and many branch points [14]. Thin fibers with more branching points are relatively resistant to fibrinolysis [15]. Conversely, if all sialic acid on fibrinogen is removed, clots form with thicker fibers and less branch points [14]. Therefore, carbohydrates can modify clot structure through both charge and mass effects on fibrin polymerization, particularly lateral aggregation [16].

Circulating fibrinogen also undergoes variable amounts of nonenzymatic glycation. Patients with uncontrolled diabetes have increased levels of fibrinogen glycation due to the high concentrations of glucose in their blood, but even nondiabetics have several sugar residues attached [17]. Higher levels of glycation appear to result in clots made up of thinner fibers and more branch points that can impact fibrinolysis potential [18, 19].

Synthesis

Fibrinogen is synthesized in the liver [20, 21] with a steady rate of production of 1.7–5.0 g per day and a large reserve capacity of up to 20-fold [22]. The polypeptide chains of fibrinogen are assembled in an ordered fashion in the rough endoplasmic reticulum [21]. Newly synthesized human B-beta chains are secreted and used more rapidly than the other two chains, which accumulate in the hepatocyte. Carbohydrate side chains are added to the B-beta and gamma chains before secretion into the plasma. Gamma chain variants are discussed later in this chapter.

Expression of mRNA for fibrinogen polypeptide chains has also been identified in non-hepatic cells such as megakaryocytes and epithelial cells in response to inflammatory mediators [23]. A

small pool of fibrinogen (but not the gamma chain variant) is stored in platelet alpha granules and appears to be taken up via GPIIb/IIIa receptor-mediated endocytosis [24].

Fibrinogen synthesis is controlled at the level of transcription [25]. The inducible component is mainly influenced by acute phase reactions mediated by interleukin-6 (IL-6), an activator of the alpha fibrinogen gene promoter [26]. Glucocorticoids and IL-6 enhance transcription of fibrinogen mRNA, whereas interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) suppress fibrinogen synthesis. This is somewhat counterintuitive since IL-1, IL-6, and TNF-alpha are all elevated during inflammation, yet they influence fibrinogen metabolism differently. Interestingly, fibrin(ogen) degradation products stimulate monocytes/macrophages to produce IL-6 [27], further upregulating fibrinogen synthesis. Fibrinogen's behavior as an acute phase reactant implicates additional stimuli and gene regulators that increase its expression; however, the control of basal transcription is required for its induction and the two cannot be separated per se. Studies in acute coronary syndromes have demonstrated that plasma fibrinogen can increase up to 20-fold with a peak elevation occurring by 3–5 days following myocardial ischemia and then gradually return to baseline following resolution of inflammation (when IL-6 levels decrease) [28, 29]. Injury is also associated with fibrinogen rebound [30] and may contribute to a post-injury hypercoagulable state. Fibrinogen synthesis also increases in response to hormonal changes during pregnancy and again in response to the injury associated with birth [31].

Alternative splicing results in a variant gamma chain, which is assembled into a normally occurring fibrinogen variant, referred to as gamma-prime fibrinogen. Approximately 8–15% of plasma fibrinogen contains a variant gamma chain (gamma-prime) [32]. The ratio of gamma-prime fibrinogen to total fibrinogen varies widely during pathologic conditions, including inflammation. The mechanism by which inflammation regulates alternative splicing leading to increase in gamma-prime fibrinogen has not been defined. This variant is associated with clots that are structurally different, with more extensive polymerization, and, importantly, removes a platelet binding site [33]. A study of patients undergoing coronary angiography has shown that levels of this variant of fibrinogen were higher in coronary artery disease patients than in patients without coronary artery disease and that this association was independent of total fibrinogen levels [34]. Although studies like these focusing on arterial thrombosis consistently demonstrate that elevated levels of gamma-prime fibrinogen are associated with cardiovascular disease, the same cannot be said of studies on venous thrombosis and thrombotic microangiopathy [35]. Some studies show a direct correlation of gamma-prime with total fibrinogen levels, whereas other studies show an inverse correlation. Furthermore, there is controversy as to whether gamma-prime fibrinogen is prothrombotic or antithrombotic. Gamma-prime fibrinogen does not lead to thrombosis in rodent models [36]. Furthermore, gamma-prime fibrinogen, compared to other fibrinogen variants, enhances the response of protein C added to plasma, decreasing thrombin generation [37]. On the other hand, gamma-prime fibrinogen has been shown to form fibrin clots with an altered clot architecture containing thinner, more numerous fibers, with more branching, that are less permeable, mechanically stronger, and highly resistant to fibrinolysis [38, 39]. Regardless of its effect on thrombosis, gamma-prime fibrinogen has consistently been associated with inflammation [40].

Metabolism

Fibrinogen has a concentration in plasma of approximately 200–400 mg/dL, with a half-life of 4 days and a catabolic rate of approximately 25% per day [41]. Fibrinogen is subject to three catabolic pathways that determine its concentration in plasma and consequently its availability to support hemostasis. These pathways are (1) basic protein turnover, (2) intravascular fibrin formation, and (3) intravascular fibrino(geno) lysis. Under physiologic conditions, coagulation and lysis account for <3% of the total catabo-

lism, and the classic catabolism pathways for most plasma proteins do not apply to fibrinogen [42]. Recently, a circadian rhythm to fibrinogen levels has been reported, with levels being highest in the morning [43, 44].

Cleavage into Fibrin

Central to the clotting mechanism is the thrombin-catalyzed conversion of soluble fibrinogen into an insoluble fibrin matrix. Fibrin polymerization is initiated by the enzymatic cleavage of the fibrinopeptides A and B located in the central E-domain of fibrinogen by thrombin, yielding a soluble fibrin monomer [45]. Fibrinopeptide A is released at a faster rate than fibrinopeptide B, which has been theorized to favor initial linear polymerization of fibrin protofibrils over lateral aggregation, thus contributing to overall fibrin structure [46]. However, as polymerization proceeds, the rate of fibrinopeptide B release increases, suggesting that either it is preferentially released from polymers or conformational changes resulting from polymerization facilitate its release [47].

Despite the small size of fibrinopeptides A and B, relative to the fibrinogen protein, their release greatly affects the solubility of the molecule. These fibrinopeptides mask complementary fibrin polymerization sites in the gamma-C and beta-C regions of the D-domains referred to as "knobs a and b" and "holes a and b" that become exposed as a result of fibrinopeptide cleavage [48–50]. Thus, given the trinodular conformation of fibrin(ogen) described earlier in this chapter, two fibrin monomers interact with each other in a half staggered fashion, in such that knob A in the E-domain interacts with *hole a* in the D-domain (Fig. 8.2) [51].

Cleavage of fibrinopeptide A alone, using viper venoms selective for fibrinopeptide A, produces thin fibers suggesting that knob A–*hole a* interactions contribute to linear fibrin monomer association and linear protofibril formation. Protofibrils also associate laterally, forming fibers. Lateral aggregation is thought to result from the D-domain interaction of two laterally aligned protofibrils [52]. The slower knob B–*hole b* interactions can contribute to lateral protofibril aggregation [46]. Although there is also evidence that alpha-C domain is another primary driver of lateral protofibril aggregation during the transition from profibril to fibrin fibers [53]. The contribution of the alpha-C regions should not be understated, given that clots missing these regions produce fibers having thin fibers and decreased tensile strength [54]. As fibers are formed through lateral aggregation, fiber branching is then necessary to produce a three-dimensional network,

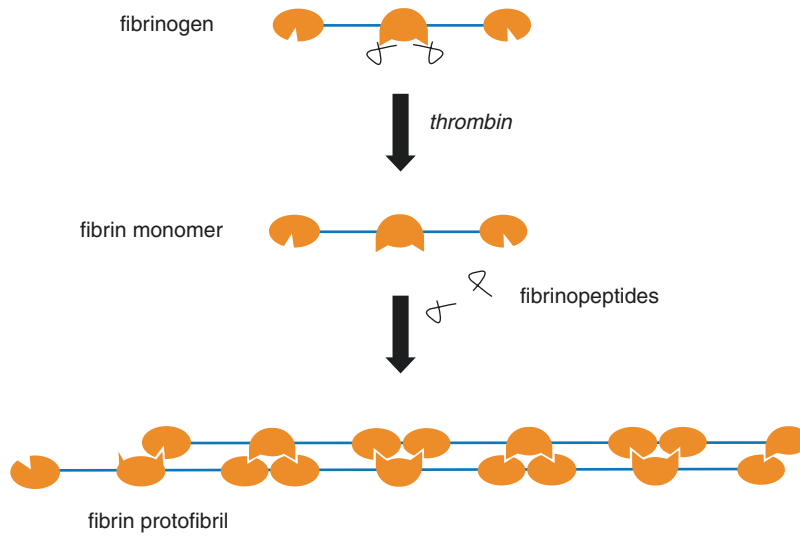


Fig. 8.2 Fibrinopeptide release and protofibril formation

leading to phase transition from a liquid to a gel. Investigations into the fractal dimension of the fibrin fiber network present at the gel point has determined that a robust fibrin formation is present prior to the gel point and correlates with subsequent mechanical properties of clots and hemostasis [55]. The gel point or its approximation can be represented clinically by measurement of clotting time in several assays [56].

Fibrin polymerization and clot structure can also be influenced by the local environment. There are four calcium binding sites in the D-domain of fibrinogen [57]. Calcium stabilizes fibrin(ogen) by giving it resistance to thermal denaturation, protecting it from reduction of disulfide bonds and from plasmin cleavage of peptide bonds [57–59]. Since the dissociation constant for calcium ion binding to fibrinogen is much lower than the free calcium ion concentration in plasma, fibrinogen calcium binding sites should be fully occupied under physiologic conditions [60]. Furthermore, calcium increases the rate and extent of lateral aggregation [60]. Generally increased overall ionic strength, and sodium ions in particular, can also reduce fibrin fiber mass/length ratio by inhibiting fibrin lateral aggregation [61].

Enhanced stiffness and durability are conferred to fibrin during its polymerization by the transglutaminase factor XIII. The affinity of factor XIII for fibrinogen (Kd 10 nM) is such that the majority of circulating factor XIII zymogen circulates with fibrinogen as a complex [62]. Interactions of fibrin with factor XIII require the presence of calcium, which is activated to its active subunits (FXIIIa) by thrombin [63]. Factor XIIIa facilitates clot stability by catalyzing the formation of γ-glutamyl-Є-lysyl amide cross-links. FXIIIa promotes both D-domain gamma–gamma cross-links and alpha-C domain cross-links that contribute enhanced overall clot stiffness, durability, and increased resistance to fibrinolysis [64]. Factor XIIIa can also participate in important protein–protein interactions involving fibrinogen and fibrin. It can cross-link alpha 2-antiplasmin, plasminogen activator inhibitor type 2 (PAI-2), and fibronectin to fibrin [65–67].

Fibrin(ogen) Interaction with Platelets and Other Cells

Fibrin(ogen) binds to integrins and other receptors on platelets and endothelial cells, serving as a bridge between cells and as a scaffold for cell migration. Fibrin also promotes inflammation and angiogenesis through interactions with leukocytes and endothelial cells. Fibrinogen binds to the activated form of the integrin $\alpha_{IIb}\beta_3$ (platelet glycoprotein GPIIb/IIIa) via residues located in the *C*-terminus of gamma chains, bridging platelets due to the dimeric nature of fibrin(ogen) [68, 69]. Figure 8.3 shows fibrin linking two platelets through binding to its GPIIb/IIIa receptor. Endothelial cells adhere to fibrin or surface-immobilized fibrinogen via the integrin $\alpha_v\beta_3$, intracellular adhesion molecule type 1 (ICAM-1) (intracellular adhesion molecule 1), and vascular endothelial cadherin [70]. Fibrin(ogen) also binds to leukocytes via the integrin $\alpha_M\beta_2$/Mac-1 which regulates inflammatory responses by localizing leukocytes to sites of injury and enabling their antimicrobial functions [71, 72].

Fibrin(ogen) and Fibrinolysis

Both fibrinogen and fibrin serve as templates for the assembly and activation of the fibrinolytic system. Plasminogen, tissue-type plasminogen activator (tPA), and alpha 2-antiplasmin all bind to fibrin(ogen). In this context, fibrin(ogen) acts as cofactor for tPA-induced plasminogen activation by their co-location (Fig. 8.4) [73–75]. Fibrin formation can increase tPA-induced conversion of plasminogen into plasmin by up to 1000-fold [5], making fibrin one of the most potent fibrinolytic activators known. Furthermore, binding of tPA to

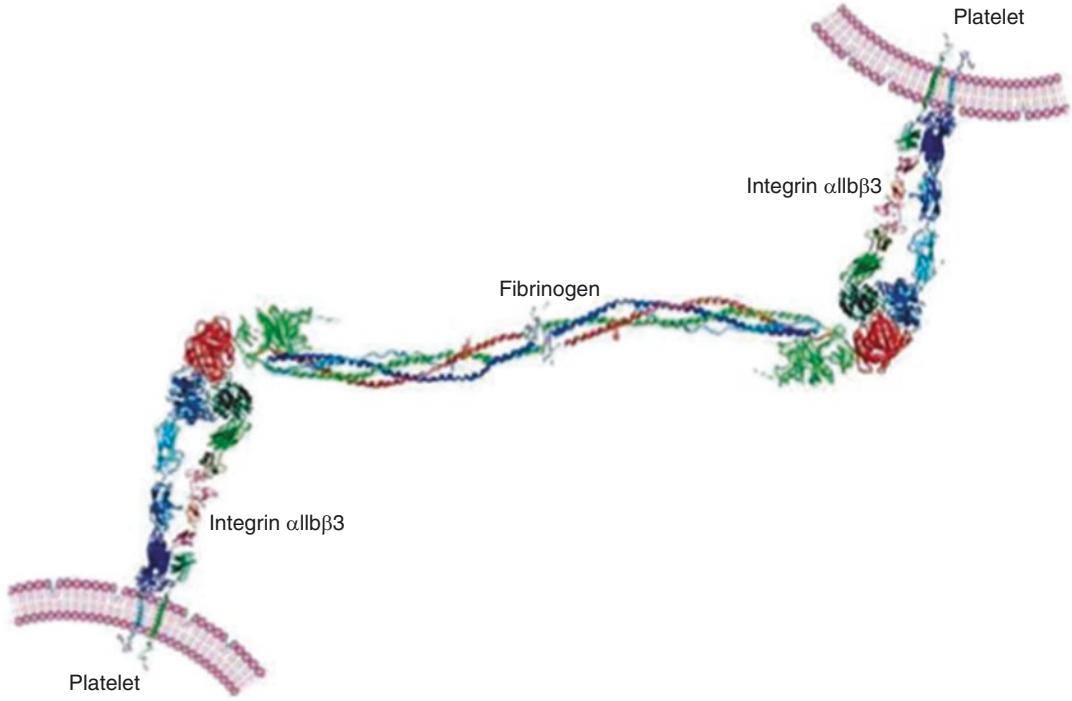


Fig. 8.3 Binding of fibrin(ogen) to two platelets through GP IIb/IIIa (integrin $\alpha_{IIb}\beta_3$) receptor

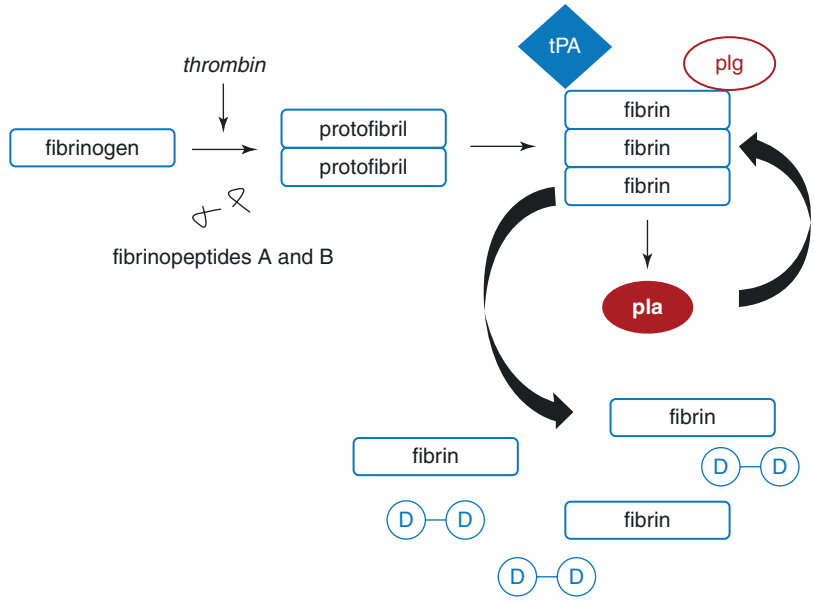


Fig. 8.4 Dual role of fibrin(ogen) in fibrinolysis: co-localizing tissue plasminogen (plg) activator (tPA) and plasminogen and serving as a substrate for the activity of plasmin (pla)

fibrin(ogen) protects it from inhibition by plasminogen activator inhibitor type 1 (PAI-1) [76].

Inherited and Acquired Fibrinogen Disorders

Disorders of fibrinogen can be classified as either type I deficiency (i.e., quantitative hypofibrinogenemia or afibrinogenemia) or type II (i.e., qualitative dysfibrinogenemia's), or some mixture of both (i.e., hypo-dysfibrinogenemia). These defects can be either inherited or acquired.

Inherited type I fibrinogen deficiencies are characterized by low or unmeasurable levels of fibrinogen protein and are typically inherited as autosomal recessive traits conferring congenital afibrinogenemia or severe hypofibrinogenemia [77]. Inherited type II deficiencies may show altered antigen levels, but predominantly have decreased coagulant activity and are more typically autosomal dominant [78]. These defects can arise from alterations of fibrinopeptide release, fibrin polymerization, fibrin cross-linking, or fibrinolysis depending upon the specific chain affected and type of mutation. Phenotypes can be asymptomatic, prothrombotic, or hemorrhagic. Fibrinogen Detroit is an example of a congenital dysfibrinogenemia exhibiting a hemorrhagic phenotype. Congenital fibrinogen disorders are named for their place of discovery in keeping with conventions similar to the one used for the description of hemoglobinopathies. Fibrinogen Detroit is characterized by an arginine→serine mutation in the N-terminal disulfide knot of the alpha chain which is believed to affect fibrin polymerization and carbohydrate content [78]. The hemorrhagic phenotype is supported by a prolonged clot initiation time, slow clotting kinetics, and reduced clot strength when examined using thrombelastography. In contrast, fibrinogen Philadelphia is a hypo-dysfibrinogenemia that is characterized by both altered fibrin polymerization and increased catabolism leading to suppressed overall antigenic protein concentration and a hemorrhagic tendency [79].

Liver disease is among the most common causes of acquired dysfibrinogenemia. It is observed in the majority of patients with cirrhosis, acute or chronic hepatitis, and also those with metastatic disease to the liver [80, 81]. Fibrinogen dysfunction in this setting is manifested by prolongation of thrombin and reptilase times but not necessarily a bleeding tendency. The abnormal fibrinogen function is attributed to an increased content of sialic acid content because sialic acid removal normalizes the thrombin time and corrects the polymerization defect [14]. Dysfibrinogenemia is also detected in the setting of renal cell carcinoma [82] and biliary obstruction [83]. Dysfibrinogenemia can also be induced by direct posttranslational modification of normal fibrinogen by its oxidation and/or nitration [84]. As reviewed by Martinez et al., oxidized histidine, methionine sulfoxide, and nitrotyrosine modifications have been documented in multiple regions of fibrinogen and correlate with changes in fibrin polymerization, clot turbidity, clot lysis, and viscoelastic properties [84]. These modifications have been documented after photooxidation for viral inactivation [85], in smokers [86, 87], in coronary artery disease [88], and in coagulopathic trauma patients [89].

There also exists a strong positive association between functional fibrinogen concentration and increased risk of thrombotic cardiovascular disease [90]. In addition, hyperfunctional fibrinogen has been detected following myocardial infarction [91]. Increased functional fibrinogen can contribute to resistance to fibrinolysis, which is a risk factor for cardiovascular disease and thrombosis [92]. Fibrinolytic resistance can arise from abnormal clot structure, high fibrinogen concentrations [93], or because of fibrinogen variants that yield fibrin clots with varying densities [94, 95]. In the later stages of trauma care, fibrinolytic resis-

tance has been associated with increased delayed mortality [96–98]. However, the direct contribution of fibrinogen quantity and function to fibrinolytic resistance after thrombosis and injury remains to be elucidated.

Fibrinogen in Trauma-Induced Coagulopathy

Fibrinogen plays a central role in trauma-induced coagulopathy (TIC) and is among the first coagulation proteins to reach critically low levels during surgical bleeding [99]. Its rapid decline after injury is perplexing because fibrinogen is present in the highest concentration of all coagulation factors. However, reasons for its rapid decline become more evident when considering the multiple ways that TIC can influence fibrinogen. TIC is associated with hemorrhage, acidosis, hypothermia, and proteolysis, each of which can contribute to changes in fibrinogen concentration and function in unique ways (Fig. 8.5).

Hemorrhage

Fibrinogen becomes limited earlier than other measured coagulation factors in both clinical studies and experimental models of hemorrhagic shock. Hiipala et al. studied 60 patients undergoing elective urologic or abdominal surgery anticipated to have blood loss exceeding 20% of a calculated total blood volume [99]. These investigators quantified fibrinogen (optical density test based on the prothrombin test), platelet count, prothrombin, and factors V and VII (one-stage test based on the prothrombin test), and blood loss was replaced using red blood cell concentrates. A critical fibrinogen concentration of 100 mg/dL was reached first when the blood loss was 1.42 times the calculated blood volume, compared to critical levels of platelet count (50,000/mm^3), prothrombin (20% activity), and factors V (25% activity) and VII (20% activity) when blood loss was 2.0 times the calculated blood volume. Using a mathematical model to analyze hemostasis during blood loss, Singbartl et al. demonstrated that blood loss is highly dependent on fibrinogen levels [100]. For a constant hematocrit (45%) and platelet count (225,000/mm^3), varying the fibrinogen concentration to 450–200 mg/dL resulted in blood loss ranging from 750 to 3750 ml.

Rapid fibrinogen loss during hemorrhage can also result from loss of blood with replacement of fibrinogen-poor resuscitation fluids/colloids [101] and/or and changes in its metabolism. To investigate the effects of moderate and severe hemorrhage on fibrinogen metabolism, Martini et al. used a swine model of hemorrhagic shock using isotopic enrichment of fibrinogen [102, 103]. Fibrinogen breakdown was accelerated compared to the control group, with no change in the rate of fibrinogen synthesis. The authors also reported that resuscitation with lactated Ringer's compared to normal saline had no differential impact on albumin or fibrinogen metabolism. Further work by the same authors investigating the effects of hypothermia and acidosis on fibrinogen metabolism revealed that hypothermia decreased fibrinogen synthesis, with no effect on fibrinogen

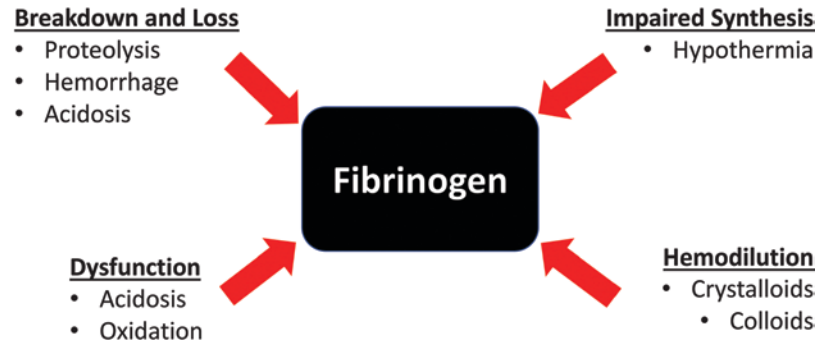


Fig. 8.5 Multiple ways that trauma-induced coagulopathy contributes to fibrinogen depletion and dysfunction

breakdown [104], whereas acidosis achieved by infusion of hydrochloric acid to a target pH of 7.1 demonstrated increased fibrinogen breakdown with no effects on fibrinogen synthesis [105]. In total, these preclinical studies support acute changes in fibrinogen metabolism that occur in response to hemorrhage, acidosis, and hypothermia that include an overall increase of fibrinogen breakdown with impaired synthesis.

Proteolysis

Enzymatic proteolysis of fibrinogen is also an important contributor to the rapid decline of fibrinogen after trauma. Tissue plasminogen activator (tPA) is elevated acutely in severely injured trauma patients and can overwhelm innate antifibrinolytic mechanisms [106]. Fibrinogen is degraded in a predictable manner by plasmin. Cleavage of both A-alpha and C-domains creates fragment X (D-E-D) [107], which remains clottable by thrombin. Further proteolysis produces the degradation product (D-E) and finally individual (D) and (E) fragments which are no longer clottable and classified into fractions A–E [107]. The presence of increasing concentrations of fibrinogen degradation products is important because fragment X has abnormal polymerization characteristics and can increase plasmin generation by provide alternative binding surfaces for tPA-induced plasmin activation (AR392-610 fragment) [108, 109].

Fibrinolysis can be inhibited by lysine and its analogues [110]. The antifibrinolytic drugs aminocaproic acid and tranexamic acid are examples of lysine analogues that are used clinically to prevent and treat fibrinolysis. These drugs work by occupying lysine binding sites on tPA, plasminogen, and plasmin, preventing their direct binding to fibrin via kringle domain interactions [110–112]. In contrast to fibrin, lysine analogues cannot fully inhibit fibrinogen proteolysis by plasmin because they do not directly occupy or inhibit its catalytic site. As a result, lysine analogues have very little, if any, effect on plasmin-induced formation of fragment X from fibrinogen. This is because plasmin–alpha-C domain interactions are not dependent upon plasmin kringle–fibrinogen interactions [113].

Fibrinogenolysis after trauma is detected by the appearance of elevated fibrinogen degradation products (FgDPs) in plasma. Retrospective studies have shown that FgDPs are associated with increased injury severity, mortality, and increased incidence of massive transfusion after trauma [114, 115]. Increased FgDP/D-dimer ratio, a more specific measure of fibrinogenolysis, is also elevated in coagulopathic trauma patients and is associated with blood transfusion needs and mortality [116]. Fibrinogen-specific degradation products may serve important roles in the pathophysiology of TIC and are promising biomarkers, but further prospective studies are required to evaluate their clinical utility.

Fibrinogen Concentration

Given the multiple effects of TIC on fibrinogen, its concentration in blood is a strong clinical indicator of the presence of TIC and predictor of transfusion needs, morbidity, and mortality after trauma [117–119]. Historical critical fibrinogen replacement thresholds of 100 mg/dl (1.0 g/L) for trauma care have been steadily increased over time [120]. More recently, admission fibrinogen concentration of <150 mg/dl was associated with increased mortality for trauma patients receiving massive transfusion [121]. The concentration threshold associated with increased general mortality after trauma has also increased to 229 mg/dl (2.29 g/L) in a multicenter study [122]. Current treatment guidelines suggest maintaining a fibrinogen concentration of at least 150 mg/dl (1.5 g/L) during trauma resuscitation [123].

Contribution to Clot Strength

One reason for fibrinogen's importance during trauma care is its contribution to whole blood clot formation. Fibrinogen is positively associated with increased whole blood clot stiffness when

measured using viscoelastic hemostatic assays [124] and is a determinant of viscoelastic clot strength in emergency department trauma patients [125]. Variants of whole blood viscoelastic hemostatic assays, including thrombelastography (TEG) and rotational thromboelastometry (ROTEM), have been developed to specifically identify the contribution of fibrinogen to clot strength [126, 127]. Harr et al. performed the TEG functional fibrinogen assay which uses abciximab, a platelet GPIIb/IIIa inhibitor, to isolate the fibrin-specific contribution to clot strength [126]. The mean contribution of fibrinogen to overall clot strength was 30%. The TEG-based fibrinogen estimates also correlated strongly (R = 0.87) with fibrinogen measured by the Clauss method. Similar methods used by ROTEM, using cytochalasin-D, an inhibitor of platelet cytoskeletal contraction, have also identified fibrin-specific clot firmness thresholds (7–10 mm) that are associated with mortality and increased blood product transfusion requirements after trauma [128, 129]. However, care must be taken to consider platelet count and the pharmacological method of platelet inhibition because of considerable variation in the degree of platelet inhibition in various assays [127].

Future Directions

Rapidly measuring fibrinogen concentration during trauma care has taken on new importance. Several methods are in development to more rapidly measure fibrinogen concentration directly in whole blood [130], and rapid estimation of fibrinogen concentration from standard measurements of hemoglobin and base excess is feasible when laboratory testing is unavailable [131].

There is also considerable interest in using fibrinogen-rich resuscitation fluids for bleeding trauma patients. Cryoprecipitate or fibrinogen concentrates are typically used to provide high-dose fibrinogen replacement. Fibrinogen concentrate can improve thrombin generation and ROTEM parameters and decrease blood loss when used in porcine trauma models [132–134]. Curry et al. tested the feasibility of using early cryoprecipitate during trauma resuscitation in an unblinded prospective randomized controlled trial [135]. They found that early protocolized cryoprecipitate increased fibrinogen concentration significantly without an effect on mortality. Treatment with cryoprecipitate (average time to treatment of 60 min) was also faster with protocolization but continued to suffer considerably from delays due to required thawing. A follow-on randomized controlled trial by the same group using fibrinogen concentrate instead of cryoprecipitate again found considerable delays to high-dose emergency therapy where one-third of patients did not receive the concentrate within the first 45 minutes of emergency care [136]. Similar to previous results using cryoprecipitate, those receiving fibrinogen concentrate demonstrated increased fibrinogen levels without an effect on mortality. However, in a prospective randomized controlled trial, Innerhofer et al. compared a fibrinogen-rich concentrate-based approach to treatment of TIC vs. a plasma-based approach and found a significant mortality benefit for concentrate-based therapy [137]. Interestingly, early high-dose fibrinogen therapy does not appear to have a significant or adverse effect on blood fibrinogen concentration during the first 7 days of trauma care [138]. Further randomized controlled studies of early high-dose fibrinogen treatment during trauma care are planned and underway and are likely to provide significant insight into the feasibility and clinical promise of high-dose fibrinogen-based therapies for trauma care [139–141].

In summary, fibrinogen is very important to TIC because of its central role in hemostasis and dynamic response to injury. Current work has elucidated much of its basic structure, metabolism, function, and interactions. Yet there remains uncertainty regarding the role of fibrinogen during TIC and its clinical utility during trauma care.

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9 Defining and Assessing the Endotheliopathy of Trauma and Its Implications on Trauma-Induced Coagulopathy and Trauma-Related Outcomes

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The Vascular Endothelial Organ and Its Role in the Regulation of Coagulation, Inflammation, and Vascular Integrity

The endothelium can be designated as an organ which communicates systemically [1]. Endothelial cells cover the entire vasculature of vertebrate animals and the surface area has been estimated to be in the range of 3000–6000 m^2 [2–4]. The total weight of the endothelium in an adult is thought to be about 720 grams mostly consisting of microvascular capillaries [2]. The endothelium communicates throughout the organism and is the platform on which coagulation, inflammation, gas exchange, control of vascular integrity, and permeability take place in both health and disease. The endothelium is strategically located at the interface between the vascular compartment and tissues, hence controlling normal blood fluidity and flow, tissue oxygenation, tissue perfusion, tissue inflammation, and targeted hemostasis. All of these areas are affected in severe hemorrhage and trauma. Further, the systemic nature of the vasculature is what makes the endothelium unique. Injury at one location affects the entire endothelial organ and targeted hemostasis, localized inflammation, and vascular leak can be compromised by the systemic nature of the insult. Based on these vital functions, it follows that the endothelium plays a central role in resultant pathology after traumatic injury.

Defining the Endotheliopathy of Trauma (EOT)

Trauma is the leading cause of death among civilians between the ages of 1 and 44 [5]. It accounts for more years of life lost than cancer, heart disease, and stroke combined, and it remains a neglected epidemic [6, 7]. Analysis of urban trauma mortality shows that almost one-third of trauma deaths are due to uncontrolled hemorrhage

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and most of these deaths occur within the first 4 h after injury [8–12]. Recent studies have identified significant early coagulopathy as a primary response to injury. This early coagulopathy occurs in 25% of injured civilian trauma patients on arrival to the hospital prior to the initiation of resuscitation and independent of hypothermia [13–15]. Further, this coagulopathy is associated with a mortality approaching 50% [14]. A subset of these patients with severe injuries develop massive activation of coagulation, producing a consumptive coagulopathy and exhaustion of the hemostatic system known as ***acute traumatic coagulopathy*** (ATC) [14, 16, 17]. This condition causes severe hyperfibrinolysis which in part is initiated by release of ***tissue plasminogen activator*** (t-PA) from damaged tissue. This release leads to the subsequent disruption of newly formed clots and tissue hypoperfusion resulting in the release of the anticoagulant activated ***protein C*** (PC) into the systemic circulation [15, 18–20]. The consequences of ATC are further exacerbated by the iatrogenic effects of hemodilution, acidosis, and hypothermia from aggressive fluid resuscitation, leading to the development *of* ***trauma-induced coagulopathy*** (TIC) [16, 18, 21–24]. The diagnosis of TIC is associated with a fivefold increase in the risk of death and makes surgical control of hemorrhage difficult [14, 15, 17, 19, 25–30]. The resulting imbalance between the activation of pro- and anti-inflammatory cascades after injury appears to contribute to the development of acute respiratory distress syndrome, multiple organ failure, and death in the intensive care unit later in the course. Optimal early transfusion strategies to treat hemorrhagic shock, prevent TIC, suppress dysfunctional inflammation, restore the damaged endothelium, and reduce oxidative stress could substantially reduce mortality and improve outcomes in bleeding patients [17, 21, 22, 28].

Resuscitation practice with balanced ratios of blood products or whole blood in severe hemorrhage and traumatic injury has more recently been optimized and implemented into practice worldwide to improve outcomes in massively transfused trauma victims [19, 31]. Clinical data supporting balanced ratios of ***fresh frozen plasma*** (FFP) to platelets to ***packed red blood cells*** (PRBCs) emerged largely from the wars in Iraq and Afghanistan where crystalloid fluids were deemed to be suboptimal. Although crystalloid fluid-based resuscitation leads to an acute restoration of vascular volume, in later stages of traumatic vascular compromise, tissue edema, and ultimate organ failure ensue [31, 32]. Damage control resuscitation and balanced ratios of blood products demonstrated improved survival in both retrospective and prospective randomized controlled trials [19, 31–33].

While much of the initial study of traumatic injury has focused on coagulopathy, hemorrhage, and treatment with transfusion resuscitation, the role of the endothelium and the interconnectedness of the endothelial, hemostatic, and inflammatory systems has become an increasing area of interest. The ***endotheliopathy of trauma*** (EOT) is a relatively new term that has been coined to encompass the combination of endothelial dysfunction, aberrant coagulation, and inflammation, all of which ensue after severe trauma and hemorrhage [34]. The relationships that encompass EOT have yet to be wholly elucidated in trauma. However, recent advances in trauma-based resuscitation as well as mechanistic understanding of novel blood products and adjuncts in resuscitation have shed light on key drivers and modulators EOT.

Understanding EOT from the Study of Novel and Standard Blood Product Resuscitation in Trauma Patients

Most trauma patients require volume resuscitation as a result of blood loss [21, 35–37]. The traditional treatment of trauma patients with hemorrhagic shock and continuing uncontrolled bleeding is prompt aggressive resuscitation with crystalloid solutions to restore effective circulating volume and systolic blood pressure following injury [35, 38–40]. However, this displaces established clots, increases inflammation, increases vascular leak and tissue edema, potentiates the development of TIC, complicates surgical

management, and dramatically increases the risk for ongoing bleeding and death [41]. To effectively correct for coagulopathy and prevent the development of TIC while restoring blood volume, the transfusion of whole blood or blood components (FFP, platelets, and PRBCs,) in a balanced 1:1:1 ratio is ideal [42].

Transfusion of PRBCs without plasma or platelets can cause coagulopathy through a number of different mechanisms [43–46]. Citrate in stored units binds endogenous calcium which acts as an anticoagulant. In patients undergoing massive transfusion, subsequent hypocalcemia can significantly alter hemostasis and hemodynamics because calcium plays a key role in the coagulation cascade and cellular metabolism [19, 47]. Storage of PRBCs causes biochemical changes that include a fall in pH to as low as 6.3 and an increase in potassium to 76 mEq/L at 35 days [48, 49]. Though patients are not exposed to such extremes, even small biochemical perturbations can affect the endothelium, hemostasis, and cellular function. In addition, administration of large volumes of red cells devoid of plasma or platelets can lead to a dilutional coagulopathy after replacement of as few as 0.5 blood volumes [13, 16, 18, 28, 50]. This necessitates subsequent administration of FFP, platelets, and cryoprecipitate to maintain normal or near-normal hemostasis [15, 26, 28, 51].

There is evidence from both military and civilian sources that resuscitation with high ratios of FFP to PRBCs results in improved survival in patients who undergo massive transfusion. The greatest benefit is seen when a high ratio is achieved within the first 6 h after injury [15, 19, 25, 26, 29, 30, 44, 51–55]. The exact etiology of this benefit is unknown, but it is presumed to be secondary to early correction of ATC and avoidance of further exacerbation of the hypocoagulable state [56–59]. ***The Prospective Observational Multi-Center Massive Transfusion Trial*** (PROMMT), performed in 10 United States Level 1 trauma centers, revealed that a 1:1 ratio of FFP to PRBCs was achieved in only 35% of patients by 2.6 h correlating to the median time to hemorrhagic death [33]. This is due to the logistical challenges associated with transfusing large volumes of FFP encountered even in large US trauma centers. Until recently, the prevention and treatment of ATC and TIC has been relatively neglected due to the prevailing concept that both are a by-product of resuscitation, hemodilution, acidosis, and hypothermia [13, 14, 18, 24, 29, 50, 60, 61]. Apart from correction of hypothermia and bleeding control, current management of coagulopathy-related bleeding is mainly based on transfusion of FFP and other blood products [16, 17, 26, 28, 30, 44]. Based on retrospective data, the Joint Theater Trauma System clinical practice guideline for damage control resuscitation in Level IIb and Level III treatment facilities in combat zones outlines the transfusion of FFP, platelets, and PRBCs in a 1:1:1 ratio. This practice was further studied in the civilian setting through a prospective randomized comparison of 1:1:1 to 1:1:2 ratios in the recently published ***Pragmatic Randomized Optimal Platelet and Plasma Ratios*** (PROPPR) study [19]. This study revealed that patients who received a 1:1:1 ratio of FFP, platelets, and RBCs achieved hemostasis earlier and were at reduced risk for death from exsanguination in the first 24 h after admission [62].

While both the PROMMT and PROPPR studies defined clear clinical practice guidelines for transfusion following trauma, the reason that plasma and platelets are so vital to resuscitation practices goes beyond their effects on coagulopathy. In addition to hemodynamic instability and coagulopathy associated with severe trauma and hemorrhage, another hallmark consequence of hemorrhagic shock is the development of the EOT [63, 64]. This physiologic phenomenon is characterized by a systemic response that results in endothelial injury, endothelial barrier compromise, dysfunctional inflammation, aberrant coagulation, tissue edema, and end organ injury [65]. Recent data generated over the past 5 years have demonstrated that plasma transfusion attenuates EOT [66–68]. Along with correcting hypovolemia and coagulopathy, there is compelling evidence that the significant survival benefit of plasma resuscitation may be related to its effects on blood-organ barrier permeability, inflammation, and vascular integrity [65, 69–76]. Plasma

inhibits endothelial cell permeability and edema after traumatic injury and is associated with restoration of endothelial adherens junctions and the endothelial glycocalyx (Fig. 9.1) [73, 74, 76–78]. In mice, plasma mitigates lung edema, and in swine, it decreases blood-brain barrier permeability and the contusion volume associated with severe traumatic brain injury [71, 72, 79]. Resuscitation with plasma compared to standard of care crystalloid fluids, such as lactated Ringer's, appears to help repair the EOT, minimizing edema and avoiding the iatrogenic resuscitation injury associated with excessive crystalloid and unbalanced blood product admin-

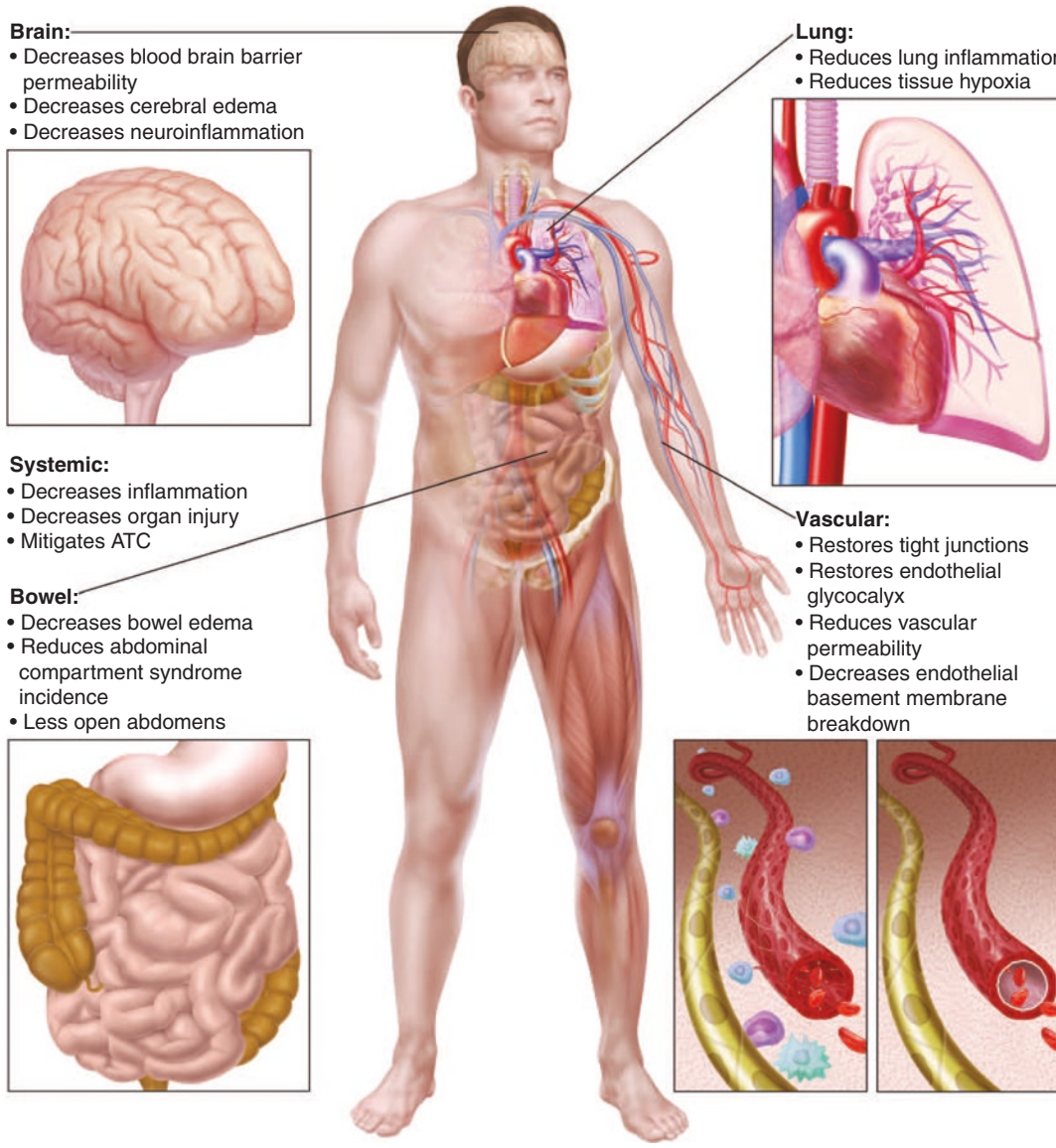


Fig. 9.1 The early use of plasma transfusion mitigates organ-specific and systemic dysfunction following trauma. Traumatic injury and resultant hemorrhagic shock results in numerous effects including a proinflammatory state, increased vascular permeability, endothelial basement membrane breakdown, exposure of subendothelial nonspecific initiation of coagulation, interstitial edema, and tissue hypoxia. Early plasma transfusion has been demonstrated to inhibit vascular permeability, mitigate acute traumatic coagulopathy, and decrease numerous organ-specific effects of injury [78]

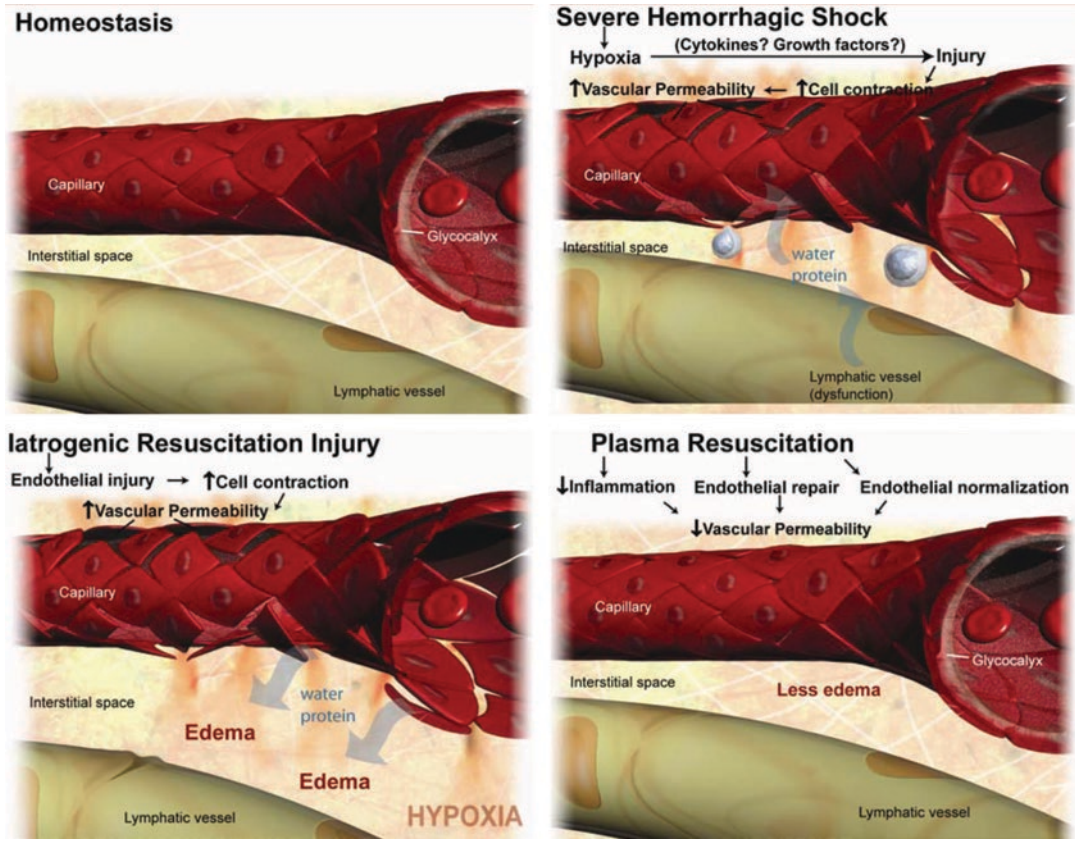


Fig. 9.2 Working model of the impact of hemorrhagic shock, iatrogenic resuscitation, and plasma resuscitation on the vasculature. Hemorrhagic shock disrupts the homeostasis of the vasculature and results in hypoxia, endothelial cell tight junction breakdown, inflammation, and leukocyte diapedesis. Iatrogenic fluid resuscitation replaces volume but does not address vascular permeability and dysfunction. FFP repairs and "normalizes" the vascular endothelium by restoring tight junctions, building the glycocalyx, and inhibiting inflammation and edema [80]

istration (Fig. 9.2) [63, 80, 81]. Similar effects have been noted for dried or lyophilized plasma and platelets [54, 58, 60, 81, 82]. While an understanding of the interconnectedness between TIC and endothelial dysfunction has emerged from preclinical studies of blood product and novel blood product transfusion in animals, but much still remains to be elucidated.

Changes in Endothelial Activation in Injury: Effects on Inflammation and Coagulation

In trauma, ***hemorrhagic shock*** (HS) is believed to be the main initiator of EOT and which can subsequently result in TIC. There have been a number of studies showing one of the first insults to the vasculature in HS, which is compromise of the ***endothelial cell glycocalyx*** (EGL), a protective border on the surface of endothelial cells [83, 84]. The EGL is located on the luminal surface of the blood vessel and is composed of a negatively charged mesh of proteoglycans, glycosaminoglycans, glycoproteins, and glycolipids which regulate leukocyte and platelet adherence to endothelial cells [87, 88]. Endothelial cells become activated in injury by loss of the glycocalyx as well as hypoxia, cytokines, and chemokines, all of which trigger innate immunity and metabolic stressors in injury [85, 86]. Studies in preclinical models have demonstrated that HS breaks down the endothelial glycocalyx, disrupts endothelial tight and adherens junctions, and

results in organ inflammation, thrombus formation, inflammatory cell activation, and organ failure [84, 89, 90]. Loss of the EGL integrity in HS and trauma increases vascular permeability leading to capillary leak (Fig. 9.2) [80]. One of the main downstream effects of EGL loss is exposure of the surface of the endothelial cells, allowing for the initiation of thrombus formation which could be nonspecific and in locations other than the injured area [91, 92]. This nontargeted injury results in clotting, inflammation, and vascular leak at sites distal to the injury which contribute to the development of TIC.

Damage and breakdown of the EGL has been linked to the deleterious effects of reperfusion in conditions such as post-cardiac arrest syndrome and to other inflammatory states, including damage caused to the endothelial cells by hyperglycemia in diabetes mellitus [93, 94]. In addition to exposure of the endothelial cells to platelets and leukocytes, breakdown of the EGL results in loss of mechanotransduction, disturbed blood flow, altered shear stress, and wall tension [83, 91]. Therefore, EGL breakdown can be the central instigating point for EOT where the downstream effects of trauma lead to endothelial dysfunction, coagulopathy, edema, and organ dysfunction, with ultimately poor outcomes.

Endothelial cells are able to react within minutes to injury and inflammation. In addition to shedding of the EGL, endothelial cells respond to thrombin and ***tumor necrosis factor alpha*** (TNF-alpha) leading to receptor-mediated signaling. A cascade of events take place in vascular injury. At baseline homeostasis in noninjured vessels, endothelial cells act by promoting anticoagulant properties and counteracting inflammation, platelet activation, and aggregation. If activated by shock or vasoactive agents, endothelial cells release ***von Willebrand factor*** (vWF) that promotes binding of platelets and t-PA that enhances control of fibrinolysis. In addition, vasoconstriction is an initial response when the vessel is damaged. It is caused by access of the smooth muscle cells to locally generated vasoactive agents and by bypassing the vasodilatory action of endothelial cells [95, 96].

There are main areas of control of endothelial cells on the clotting cascade and thrombus formation in hemostasis and the development of TIC. It is beyond the scope of this review to get into the details of clotting at the surface of the endothelial cell platform; however, the interconnectedness between these components and the development of EOT and TIC have yet to be elucidated. The control of coagulation involves aspects such as binding of antithrombin III, thrombomodulin expression, PC activation, expression of ***endothelial protein C receptor*** (EPCR), and release of the ***tissue factor pathway inhibitor*** (TFPI) [92, 97]. At the level of platelet adhesion and activation, hemostatic control is maintained by the production and release of vWF as well as cleavage of ultralarge vWF by ADAMTS13 [97, 98]. Fibrinolysis has control mechanisms based on the release of t-PA, induction of plasminogen activator inhibitor-1, and stimulation of urokinase-type plasminogen activator along with its receptor [99–101]. Platelet disintegration involves ADAMTS18 [102]. These are just a few aspects of hemostatic control at the level of the endothelial cell.

In addition, there are also important connections between clotting and endothelial cell permeability. One of the main pathways is triggered by thrombin which is a large modulator of vascular leak. The pathway first involves the physiological activation of protein C by the thrombin IIa-thrombomodulin complex on the surface of endothelial cells which is facilitated by the EPCR. Activated PC exerts its anticoagulant effects by proteolytic inactivation of factors Va and VIIIa [103]. In addition, activated PC associated with EPCR results in cleavage of protease-activated receptor 1 to initiate cell signaling with cytoprotective effects that may include anti-inflammatory and antiapoptotic activities, altered gene expression profiles, and barrier protective effects [103, 104].

Current Strategies for Clinical Evaluation of EOT in TIC

One of the many enigmas of TIC are the key characteristics of patients at risk including how an individual's vascular endothelium may play a

role in the development of TIC independent from the degree of blood loss and HS status. Coagulopathy plays an important role in trauma-related morbidity and mortality both due to its role in hemorrhage and as a marker of endothelial dysfunction. TIC is a multi-phenotypic and phasic disease process that is not only indicative of impaired hemostasis, but is also connected to disordered inflammation, endothelial permeability, and overall vascular homeostasis [79, 105, 106]. As understanding of TIC has grown, so has an appreciation for the important interaction of the hemostatic and endothelial systems in trauma as well as the impact of hemorrhagic shock on vascular homeostasis. EOT is an emerging area of clinical study and has the potential to unify mechanisms underlying TIC as well as generate targeted pathways for therapy [34].

Given the evolving importance of EOT, many studies in recent years have sought to define EOT and markers of endothelial dysfunction clinically in trauma patients. Table 9.1 describes key clinical studies elucidating the presence and consequences of endotheliopathy following traumatic injury. Much of this work has focused on serum measurement of biomarkers of glycocalyx degradation, cellular injury, and vascular leak and subsequently correlating these measures to both markers of coagulopathy and clinical outcomes. Specifically, syndecan-1, thought to be a measure of endothelial glycocalyx shedding, has been associated with coagulopathy on viscoelastic

Table 9.1 Summary of key clinical studies investigating the presence of endotheliopathy following trauma. All studies were single-center studies conducted at Level 1 Trauma Centers

Study	Patients investigated	Key findings
Ganter et al. [117]	208	Angiopoiten-2 release was associated with: Injury severity and global hypoperfusion Von Willebrand factor release, soluble thrombomodulin elevation, coagulopathy, and activation of the compliment cascade Worse clinical outcomes including mortality, length of time on a ventilator, acute renal failure, and transfusion need
Ostrowski et al. [107]	77	Patients with autoheparinization determined by the difference in kaolinTEG and heparinaseTEG had fourfold higher syndecan-1 levels, higher international normalized ratios, higher thrombomodulin, higher interleukin 6, and lower protein C indicating the presence of endothelial damage, coagulopathy, and inflammation
Ostrowski et al. [108]	404	Soluble VE-cadherin and syndecan-1 were associated with hypocoagulable rTEG along with high plasma adrenaline, male sex, high injury severity, low platelet count, and prehospital transfusion
Johansson et al. [109]	424	Adrenaline was an independent predictor of syndcan-1 level Adrenaline and sydecan-1 were independent predictors of <24-h, 7-day and 28-day mortality Thrombomodulin was an independent predictor of 7-day and 28-day mortality
Gonzalez Rodriguez et al. [23]	410	A syndecan-1 level 40 ng/mL or greater was associated with 30-day in-hospital mortality and need for transfusion
Naumann et al. [119]	122	Cell-free DNA levels were associated with syndecan-1 and thrombomodulin levels in a linear relationship Patient mortality was associated with higher prehospital and in-hospital cell-free DNA levels
Naumann et al. [90]	17	Syndecan-1 and thrombomodulin elevations were associated with worse flow, density, and heterogeneity within microvessels after traumatic hemorrhagic shock within 10 h of injury as measured by sublingual incident dark-field video-microscopy
Naumann et al. [110]	91	Endothelial dysfunction measured by elevated syndecan-1 and thrombomodulin was present within 60 min injury and, when elevated levels persisted at 12 h after injury, was associated with organ dysfunction

(continued)

Table 9.1 (continued)

Study	Patients investigated	Key findings
Gonzalez Rodriguez et al. [111]	258	Patients with a serum albumin of <3.6 g/dL were more likely to have: Endotheliopathy defined by a serum syndecan-1 level of 40 mg/mL or greater Worse outcomes including more frequent blood transfusions, fewer hospital-free days, and higher mortality rates
Wade et al. [120]	24	Patients with endotheliopathy based on elevated syndecan-1 had reductions in clot initiation, amplification, propagation, and strength on thrombelastography There were no differences in endothelial microvesicles, plasma norepinephrine, soluble E-selectin, soluble VE-cadherin, and histone-complexed DNA fragments levels in those with and without endotheliopathy
Richter et al. [118]	52	Plasma Ang-1 and Ang-2 are elevated immediately after pediatric trauma compared to controls At 24 h, pediatric trauma patients had elevated plasma Ang-2/Ang-1 ratios relative to controls due to decline of Ang-1 levels to near that of controls Higher Ang-2:Ang-1 ratio was associated with poor outcomes Ang-2 was associated with more hypoperfusion and syndecan-1

testing, inflammation, hypoperfusion, morbidities including transfusion need and longer hospitalization, and mortality [90, 107–111]. Syndecan-1 is involved in several of the downstream effects following traumatic injury that lead to the EOT [84, 107, 108, 112, 113]. Syndecan-1 has been touted as a prime clinical biomarker to depict outcomes in trauma patients. However, it may have been overly emphasized as a measure of endothelial compromise and dysfunction considering a major source of syndecan-1 is the bronchoalveolar epithelium [114, 115]. The lungs are often the first organ to display or manifest the effects of severe trauma and hemorrhage and it can be speculated that the majority of the circulating syndecans are from epithelial cellular injury. Hence, syndecan-1 is a marker of global injury but it is not likely a specific marker of endothelial injury [116].

Activation of the endothelium measured by ***angiopoiten-1*** (Ang-1) and ***angiopoiten-2*** (Ang-2) has also been associated with coagulopathy, inflammation, morbidity, and mortality [117, 118]. Some authors have suggested that endothelial integrity and permeability are regulated through direct cellular damage as measured by elevated soluble VE-cadherin and an elevated Ang-2 to Ang-1 ratio [108, 118]. This is supported by Naumann et al. who found that cell-free DNA, a marker of cellular death, was associated with elevated syndecan-1, thrombomodulin, and mortality [119]. Interestingly, Wade et al. found somewhat contradictory results with no evidence of direct endothelial injury after trauma. This suggests that increased endothelial permeability may occur from mechanisms other than direct cellular damage [120].

Thrombomodulin is another possible clinical marker that has been utilized as a surrogate marker for injury to the vasculature. Thrombomodulin is an anticoagulant protein expressed on the surface of endothelial cells and is active in playing a role in the PC anticoagulant pathway as described above [103, 104, 121–123]. After trauma, the increase of inflammatory cytokines such as tumor necrosis factor α and interleukin 6 results in cleavage of thrombomodulin and release into the circulation as soluble thrombomodulin, a less active form and a well-known marker of endothelial cell injury [121, 122, 124]. Taken together, there are numerous clinical markers of endothelial dysfunction that have been linked to TIC and poor outcomes in trauma patients. The utility of these markers to drive decision support and therapeutics is an area of active investigation.

Finally, there are several cells and cellular components that hold promise as markers of endothelial dysfunction in trauma patients. ***Circulating endothelial cells*** (CECs) have been investigated as another surrogate for endothelial cell damage and can be readily obtained through a blood sample [125, 126]. Importantly, CECs are rarely found in the blood in healthy individuals. Due to a variety of vascular insults including trauma, endothelial cells may detach from the endothelial monolayer and these resultant CECs can be sampled and measured from a peripheral blood sample. Elevated levels of CECs are seen in a variety both acute and chronic disease [125, 127–129].

Blood outgrowth endothelial cells, also referred to as late outgrowth ***endothelial progenitor cells*** (EPCs) or endothelial colony-forming cells, are stem cells derived from bone marrow that have the ability to differentiate into mature endothelial cells. EPCs are mobilized from the bone marrow through endothelial nitric oxide synthase in response to vascular injury and need for repair and neovascularization [130, 131]. Similar to CECs, these cells have been used as a marker of molecular endothelial dysfunction and have application in revascularization in damaged tissues. Though not yet pursued in TIC or EOT, they have provided pathophysiologic insights into other endothelial-based diseases including hematologic disease such as von Willebrand disease and sickle cell disease, pulmonary arterial hypertension, ischemic heart disease, and chronic lung diseases [131–133]. Both CECs and EPCs provide the benefit of direct cellular assessment in a relatively noninvasive manner. Though protocols are still being developed and refined, enumeration of CECs may provide useful information in the monitoring of disease activity and treatment efficacy [129, 130, 133].

Finally, microparticles or extracellular vesicles are cellular components of submicron size from various cellular origins. They are shed from plasma membranes in response to cell activation, injury, or apoptosis and serve an important role in intercellular communication [134, 135]. Microparticles were in fact first described related to their procoagulant properties. Enumeration of microparticles during disease progression and in response to treatment may provide a way of assessing degree of endothelial damage. Further, several studies suggest specific phenotypes of microparticles that may alter hemostatic capacity and can predict the presence of trauma-induced coagulopathy and transfusion response [136–138].

Additional Consideration of Endothelial Heterogeneity

While evaluation of systemic biomarkers has shown diagnostic promise, additional consideration of the endothelium as a heterogeneous organ is also important. A comparison of endothelial cells cultured from arteries, veins, and microvessels from various tissues demonstrated that arteries and veins display distinct differences. Specifically, microvascular endothelial cells from microcapillaries had the greatest differences compared to other areas in the vasculature [139]. This is reflected in differential expression of microvascular surface receptors such as alpha-1, beta-1, and plasmalemma vesicle-associated protein-1 which is a leukocyte trafficking molecule [140, 141]. Tissue-specific endothelial expression of g-glutamyl-transpeptidase and monoamine oxidase has been found on brain microvessels [142, 143]. Bone marrow endothelial cells express E-selectin constitutively; however, in other cell types E-selectin requires inflammatory activation of endothelial cells. Thrombomodulin is another marker that is abundantly present on most endothelial cells but is not present on brain or liver sinusoidal endothelial cells [144]. Differences between microvascular endothelial cells have been reviewed in the heart, kidney, and lungs by Aird and colleagues [145, 146].

Timing also plays a key role in the understanding of endothelial-specific biomarkers. In the development of TIC, mortality occurs within hours after injury, and there is likely not enough time for acute gene expression alterations in the vasculature to impact outcomes. However, what likely should not be ignored is the baseline of endothelial function where the patient started

prior to the injury. For example, the status of KLF2 expression, an endothelial transcription factor that suppresses activation of endothelial cells to inflammation and thromboses, may have differential individual baseline expression that could result in different degrees of TIC and differential development of HS if that individual is exposed to injury [147, 148]. While difficult to assess, a patient-specific baseline of the endothelium prior to injury may be of critical importance in understanding how EOT manifests in injured patients.

Direct Visualization and Monitoring of the Endothelial Bed

To address evaluation of dynamic changes of the endothelium, visualization of a specific endothelial bed is an area of much development. Common clinical imaging studies such as Doppler measurements of blood flow, magnetic resonance angiography, and CT scanning are widely available and may become more applicable to endothelial dysfunction in the near future. Other imaging techniques are not actively used in the clinical assessment of trauma patients but have yielded beneficial results in other disease populations [149]. One such approach of endothelial bed assessment can be done using high-resolution external vascular ultrasound to measure changes in brachial artery diameter in response to reactive hyperemia [149, 150]. Molecular imaging, which combines the power of proteomics with advanced labeling techniques, is likely to revolutionize the diagnosis of endothelial-based disorders.

In addition, there have been attempts to establish dark-field microscopy as a method by which to assess endothelial dysfunction and measure degradation of the glycocalyx through utilization of sublingual cameras by Vink and colleagues [151, 152]. In a recent study involving septic patients, microcirculation parameters were evaluated and the EGL sublingually. It was found that the EGL was measurable and compromised in septic patients but did not correlate to microcirculatory impairment. Further studies in critically ill patients are needed to unravel the relationship of glycocalyx damage and microvascular impairment, as well as their prognostic and therapeutic importance in sepsis and other diseases such as trauma [153]. Further development is required to establish the clinical relevance of this form of assessment in evaluating and treating trauma patients.

There may be more direct and invasive measures of the endothelium on the horizon as well. For example, peripheral vascular endothelial cell biopsy is possible by inserting a catheter into a peripheral artery or vein and gently abrading the luminal surface of the vein with a J-wire to harvest them. These cells are then recovered from the wire and plated on poly-L-lysine-coated slides [154, 155]. From there, they can be treated with various therapeutic interventions and assayed for mRNA, protein expression, and functional effects [155]. Another possible approach that could be borrowed from endothelial evaluation in atherosclerosis and coronary artery disease involves placement of directed catheters that allow for examination of blood from a specific endothelial bed [156]. In this strategy, vessel diameter and blood flow characteristics can be evaluated at baseline and in response to vasodilators and modulators of permeability. In addition, endothelial physiology may be assessed by local examination of the blood for biomarkers directly released by the endothelium. While this technique provides appeal in its real-time and direct monitoring of the endothelial bed, the practically of adapting this invasive technique to the trauma population given its acuity is a significant challenge.

Finally, newer proteomic-based techniques are likely to provide novel platforms, such as protein-based chips, that will allow the practitioner to simultaneously monitor a panel of endothelial cell function biomarkers. For example, proteomics may be valuable in determining the presence or absence of specific complications such as graft versus host disease or sepsis in patients undergoing hematopoietic cell transplantation, in understanding changes in blood-brain barrier function in central nervous system disease, or in assessing the pathologic mechanisms in stroke. By extension, a more comprehensive

and systematic analysis of pathological specimens, such as skin biopsies, might provide even more robust data that reflect endothelial cell function.

Future Directions, Novel Therapeutics, and Gaps in the Study of the EOT

There are a number of new emerging areas that have potential to modulate outcomes in trauma through mitigation of the EOT. In addition to transfusion, many adjunctive therapeutics are ongoing areas of investigation. For example, valproic acid, a histone deacetylase inhibitor, has been shown to induce platelet aggregation, increase clot strength, and improve clot formation rate in animal models [157]. There are active clinical trials aiming to understand its utility in the trauma population. Tranexamic acid, a synthetic derivative of the lysine that inhibits fibrinolysis by blocking the interaction of plasminogen with the lysine residues of fibrin, has been studied for many years in the trauma population and is being newly explored to modulate endothelial compromise and clotting in specific subpopulations including traumatic brain injury patients [158].

Another burgeoning area of research involves the use of cell-based therapies as a novel approach to treating traumatic injury. These therapies are unique in that they have the ability to interact with multiple therapeutic targets including the components of EOT [159]. These pleiotropic effects may be of particular benefit in trauma patients because of the complex and heterogeneous patterns present in traumatic injury [160]. For example, cellular therapies administered after traumatic brain injury have been shown to affect blood-brain barrier permeability, production of inflammatory cytokines and chemokines, and activation of critical inflammatory cells such as microglia, neutrophils, and macrophages [160–162]. Clinical application of these therapies is ongoing in multiple venues, the results of which will likely have a significant impact on management approaches.

In sum, a key challenge in the management of trauma patients with EOT is understanding the complex interconnections between the development of TIC, vascular leak, and inflammation. Critical to advancement in this field is finding ways to measure the EOT clinically through biomarkers and newly devised measures to image and assess endothelial damage. It is only through clear mechanistic and diagnostic understanding with attention to the heterogeneity of the endothelial system, the dynamic real-time changes of that system, and the baseline factors of the individual patient can relevant therapeutic targets and ultimately improved outcomes be achieved.

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Protein C

10

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In normal human hemostasis, the activated protein C (APC) pathway attenuates coagulation, serving as an anticoagulant restraint on multiple physiologic processes that promote clot formation. Independent of these anticoagulant properties, APC has cytoprotective effects, acting as an anti-inflammatory agent and preventing endothelial barrier leakage [1, 2]. By these two mechanisms, the APC pathway serves to maintain vascular flow by preventing excessive thrombosis and to protect cells from damage associated with inflammatory insults like sepsis and trauma.

Molecular Components of the Activated Protein C Pathway

Protein C

Protein C was identified and named in Sweden in 1976 by Stenflo, who in his investigations of bovine coagulation factors found "a hitherto unrecognized vitamin K-dependent protein with an unknown function." The name was derived from its position as the third peak eluted via chromatography, peak C [3]. Protein C had, in fact, been described as "autoprothrombin IIa" the decade before in Detroit by Seegers [4, 5]. In as early as the 1960s, this protein was known to have anticoagulant properties and was thought to have an inhibitory effect on fibrinolysis [6]. Thus, the discovery of protein C occurred through laboratory efforts to define the fundamental biochemistry of coagulation in the 1960s and 1970s, prior to any awareness of its clinical importance.

Protein C was subsequently isolated and identified as a vitamin K-dependent zymogen precursor of a serine protease, the activation of which is mediated via binding of thrombin [7]. Once activated, APC achieves its primary anticoagulant function through irreversible proteolytic cleavage of activated factors Va and VIIIa, the major drivers of thrombin formation [8–11]. While these anticoagulant mechanisms were detailed thoroughly by the 1980s, the cytoprotective activity of APC, with respective mechanisms via specific receptors, was not described until decades later [12].

The structure of protein C is homologous to that of the other vitamin K-dependent coagulation proteins, from the level of nucleotide sequence to that of tertiary protein folding. The gene for protein C is located on chromosome 2, spans approximately 11 kilobases, and codes for a precursor 461-amino-acid polypeptide [13, 14]. Before secretion, this precursor is modified by removal of a signal peptide and a propeptide involved in gamma-carboxylation, yielding a 419-amino-acid product with a molecular weight of 62 kDa [15] (Fig. 10.1). An endoprotease

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PROTEIN C

-42 MWQLTSLLLFVATWGISGTPAPLDSVFSSSERAHQVLRIRKR -1

1 ANSFLEELRHSSLERECIEEICDFEEAKEIFQNVDDTLAFWSKHVDGDQC 50

51 LVLPLEHPCASLCCGHGTCIDGIGSFSCDCRSGWEGRFCQREVSFLNCSL 100

101 DNGGCTHYCLEEVGWRRCSCAPGYKLGDDLLQCHPAV KFPCGRPWKRMEK 150

151 KRSHLKRDTEDQEDQVDPRLIDGKMTRRGDSPWQVVLLDSKKKLACGAVL 200

201 IHPSWVLTAAHCMDESKKLLVRLGEYDLRRWEKWELDLDIKEVFVHPNYS 250

251 KSTTDNDIALLHLAQPATLSQTIVPICLPDSGLAERELNQAGQETLVTGW 300

301 GYHSSREKEAKRNRTFVLNFIKIPVVPHNECSEVMSNMVSENMLCAGILG 350

351 DRQDACEGDSGGPMVASFHGTWFLVGLVSWGEGCGLLHNYGVYTKVSRYL 400

401 DWIHGHIRDKEAPQKSWAP 419

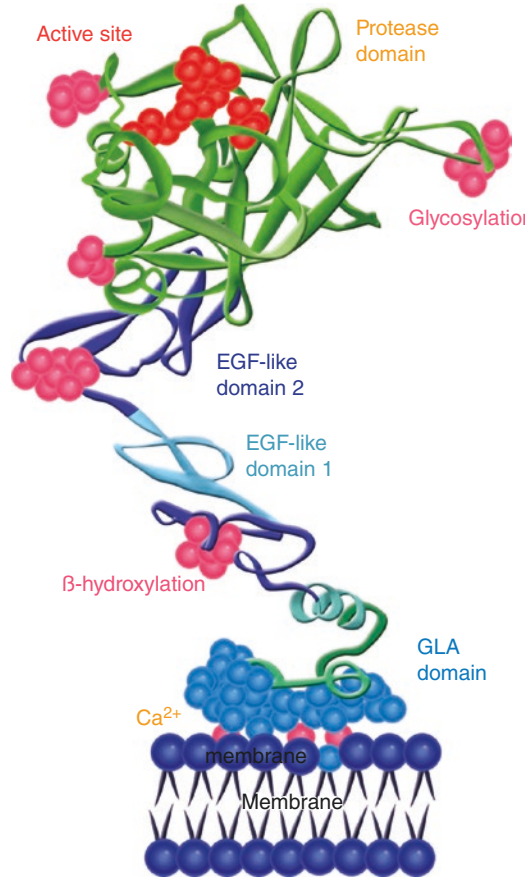


Fig. 10.1 Amino-acid sequence of protein C and ribbon polypeptide scheme of activated protein C. Amino acids are numbered from the amino-terminus of the mature protein with the signal-peptide sequence underlined. Specific domains are color-coded as indicated in the ribbon cartoon. Green circles depict γ-carboxylation, blue circles β-hydroxylation, and black circles N-linked glycosylation. Sites of proteolytic cleavage during post-translational processing and protein C activation are indicated by scissors. The dipeptide proteolytically removed during post-translational processing of most protein C molecules in the liver is identified by the box. The serine, aspartic acid, and histidine residues that constitute the active-site triad are identified by red circles. (Modified from Mosnier and Griffin [18])

cleaves the protein into a two-chain zymogen, with a heavy chain covalently linked to a light chain by a single disulfide bond. The majority of protein C is found in this form [16]. The amino-terminal gamma-carboxyglutamate (Gla) domain of the light chain enables calcium chelation and binding of membrane complexes, as in homologous vitamin K-dependent clotting factors; this domain also facilitates binding to endothelial receptors [17]. Two epidermal growth factor (EGF) domains on the light chain likely mediate interaction with cofactors such as thrombomodulin and protein S; the activation peptide and serine protease are found on the heavy chain. Post-translational N-linked glycosylation occurs prior to secretion of the mature glycoprotein. The majority of protein C is synthesized in the liver, circulates at a plasma concentration of 4 mcg/mL (70 nM), and has a half-life of 8 h [18].

Activation of the protein C zymogen is achieved via thrombin-mediated cleavage of the heavy chain at Arg169, releasing a 12-residue activation peptide [7]. This reaction is accelerated by endothelial-bound thrombomodulin and the endothelial protein C receptor (EPCR). Once activated, APC either is released as an anticoagulant or remains bound to EPCR, whereby its cytoprotective effects are initiated [19]. The serine protease domain of the APC molecule is homologous to that of chymotrypsin and mediates the highly specific inactivation of coagulation factors V and VIII [20]. Circulating APC has a half-life of approximately 20 min, and levels are low in healthy adults, on the order of 1–3 ng/mL (<40 pM) [21]. APC levels closely correlate with levels of the circulating zymogen, suggesting that precursor protein C concentration is a limiting factor in the rate of in vivo APC activation [22].

Thrombomodulin

Although thrombin is responsible for the activation of protein C, the kinetics of the thrombin-protein C reaction are extremely unfavorable. Hypothesizing that another mechanism augments this process in vivo, Esmon and Owen found that protein C activation increased 20,000-fold during exposure to endothelium in the coronary microvasculature of an explanted rabbit heart, and this effect was confirmed in cultured human endothelial cells [23]. The responsible endothelial cofactor, thrombomodulin, was subsequently isolated and named for its ability to convert thrombin from the canonical procoagulant to a functional anticoagulant via protein C activation [24].

Thrombomodulin is a transmembrane protein found predominantly on the surface of endothelial cells. It is coded on chromosome 20; has a molecular mass of approximately 70 kDa; and contains a lectin-like domain, six EGF domains, and a serine/threonine-rich domain near the membrane that serves as a site for glycosylation [25]. Thrombin and protein C bind at the EGF domains [26]. Once bound to thrombomodulin, thrombin undergoes conformational changes that affect its substrate specificity, shifting the balance from the procoagulant thrombin-fibrin pathway to the anticoagulant APC pathway [27, 28]. At the ser/thr domain, binding of glycosaminoglycans like chondroitin sulfate increases thrombomodulin's affinity for thrombin, enables further protein C activation, and accelerates thrombin inhibition by proteases [29, 30]. Thrombin bound to thrombomodulin is inactivated more rapidly than free thrombin [31].

Thrombomodulin expression differs by tissue type, and given a constant endothelial concentration, it has higher activity in the microvasculature than in larger vessels due to the several 100-fold increase in endothelial surface area-to-blood ratio in capillaries [32, 33]. Thus, it prevents thrombosis in small peripheral vessels, where thrombin activity might otherwise favor clot formation. In mice, deletion of the thrombomodulin gene is fatal at the embryonic stage [34].

Thrombomodulin has been found to circulate in plasma in soluble form, presumably following cleavage of the membrane-binding and cytoplasmic domains [35]. Elevated levels of soluble thrombomodulin have been associated with certain pathologic states, including atherosclerosis and sepsis [36, 37]. The functional relevance of this circulating pool is unclear. While it likely represents a marker of endothelial damage, especially when elevated above normal levels [38],

studies have shown that it retains function as a cofactor in protein C activation [39]. More recent data indicate that soluble thrombomodulin released during endothelial stress might have a protective role in inflammatory conditions, exerting an antiapoptotic effect on target cells [40].

Protein S

Protein S was discovered in 1979 by DiScipio and Davie at the University of Washington and was named for the city of Seattle. It was initially identified as a vitamin K-dependent plasma protein with unknown function [41] but was soon found to be a potent cofactor with APC in the inactivation of factor Va [42].

Protein S is a 77-kDa glycoprotein coded on chromosome 3. After the removal of a leader peptide, it is secreted as a single-chain polypeptide of 635 amino acids with three carbohydrate side chains [43, 44]. Like homologous vitamin K-dependent glycoprotein relatives, it has a Gla domain that binds calcium and facilitates binding to phospholipid membranes. Following a thrombin-sensitive domain are four EGF-like domains, which have been implicated in its cofactor activity [45]. However, protein S is not a serine protease and circulates in active form. A C-terminal sex hormone binding globulin (SHBG)-like domain facilitates binding to C4b-binding protein (C4BP), a regulatory glycoprotein of the complement system [46]. Approximately 60% of circulating plasma protein S is bound to C4BP, and this bound form has reduced APC cofactor activity. The remaining free 40% (~25 μg/mL, ~130 nM) of protein S is available as an active cofactor [47, 48]. While primarily synthesized in the liver and endothelium, protein S has been shown to be produced by other cell types, including activated platelets [49].

Early work identified the role of protein S in facilitating binding of the APC complex to the phospholipid membrane, enabling inactivation of factor Va [50]. Multiple protein S domains are necessary for effective augmentation of APC activity, with recent studies implicating the first EGF-like domain as the critical site for interaction with APC [45]. A Gla domain has also proven key to effective interaction and potentiation [51]. Via these mechanisms, protein S helps to bind APC to the phospholipid membrane in proximity to Va and VIIIa and orients the structure of the enzyme-substrate complex to optimize proteolysis [52], increasing inactivation of Va up to 20-fold [53] and inactivation of VIIIa at least threefold [54].

Independent of its role as a cofactor, protein S can function as a direct inhibitor of thrombin formation via a host of proposed mechanisms, including direct binding and blockade of coagulation factors and potentiation of tissue factor pathway inhibitor (TFPI) [55, 56]. Studies have also identified a separate cytoprotective effect, whereby protein S facilitates clearance of apoptotic cells via specific cell-surface receptors [57].

Endothelial Protein C Receptor

In an effort to understand the endothelium-mediated process of protein C activation and the suspected role of APC in modulating inflammatory responses, the Esmon lab identified and characterized the endothelial protein C receptor in 1994 [58]. Subsequent studies demonstrated that EPCR increased protein C activation fivefold in vitro [59]; in vivo primate studies indicated a 20-fold enhancement of protein C activation via EPCR [60].

EPCR shares structural homology with the major histocompatibility class I (MHC I) or CD1 superfamily of transmembrane receptors. The 46,000-kDa mature glycoprotein contains 221 amino acids and is coded on chromosome 20q [61]. In the site where MHC molecules contain a presented antigen, EPCR is tightly bound to a phospholipid, the removal of which eliminates binding to protein C [62]. EPCR binds to the Gla domains of protein C and APC with similar affinity [17]. By binding the zymogen protein C and presenting it to the thrombin-thrombomodulin enzymatic complex, EPCR reduces the substrate concentration required for effective activation, which is otherwise greater than the normal plasma concentration [63]. Via binding to EPCR, APC exerts its cytoprotective effects through

cleavage of the PAR-1 receptor [12]. Although originally identified on human endothelium, EPCR has been localized to other cell types, including hematopoietic, epithelial, and neuronal cells. It has also been shown to have ligands other than protein C and seems to play a complex role in the pathogenesis of inflammation, cancer, and severe malaria [64]. Unlike thrombomodulin, which is found at highest concentrations in the microvasculature, EPCR is more densely concentrated in larger blood vessels [33].

A soluble form of EPCR, which circulates in normal plasma at a concentration of 100 ng/mL (~2.5 nM), has been shown to bind protein C with an affinity similar to the membrane-bound form [65]. This soluble form inhibits the anticoagulant properties of APC in vitro both by blocking membrane binding and by inducing structural changes in the APC-binding site [66]. Soluble EPCR is increased in the settings of sepsis and systemic lupus erythematosus [67] and elevated in trauma patients with critical injuries [68]. EPCR release from the endothelium has been related to tissue necrosis factor alpha (TNF-α) and circulating thrombin [69]. As with thrombomodulin, the meaning of elevated soluble EPCR in these disease states remains unknown, possibly representing endothelial-membrane shedding and nonfunction or increased expression and function. However, based on murine models, the effect of soluble EPCR on systemic coagulation might be insignificant at physiologic levels [70].

Pathway Regulation

For protein C to be activated, thrombin binds endothelial thrombomodulin as it is generated, forming an enzymatic complex on the endothelial surface. Zymogenic protein C binds EPCR at the Gla domain and is oriented to the thrombin-thrombomodulin complex for activation. Thrombin cleaves the zymogen at the Arg169-Leu170 bond, producing the functional protease APC. This APC can release from EPCR to bind protein S in the anticoagulant pathway, or it can remain bound, whereby its cytoprotective actions are mediated [71].

One known stimulant of protein C activation is platelet factor 4, a cationic alpha-granule protein released by activated platelets in the presence of damaged endothelium. Initially found in vitro to increase protein C activation by up to 25-fold in a Gla-domain-dependent manner [72], platelet factor 4 infusion also produced elevated APC levels in a primate in vivo model, presumably by enhancing the affinity of protein C for the thrombin-thrombomodulin complex [73]. Earlier studies showed that protein C activation was also accelerated by factor Va, both in solution and on endothelial cells [74]. Negatively charged endothelial phospholipids, which can be exposed after thrombin-induced cell activation, significantly increase APC formation; this effect could be mediated via a calcium-dependent interaction with protein C [75].

Generation of APC is limited by zymogen availability, measured by circulating protein C level [76], and thrombin generation. For similar reasons, given the strong dependence of protein C activation on thrombomodulin and EPCR, changes in the expression or activity of these components represent a key means of altering APC production. For example, inflammatory processes reduce protein C activation by a host of mechanisms: TNF can downregulate thrombomodulin and EPCR transcription [58, 77], homocysteine has been shown to inhibit thrombomodulin directly and reduce its cell-surface expression [78], and atherosclerosis decreases thrombomodulin and EPCR expression on the endothelial surface [79]. Proteolytic release of thrombomodulin from the cell surface is another mechanism for downregulation and has been associated with neutrophil elastase [80]. Interestingly, heat shock has an opposing effect, increasing the transcription of thrombomodulin in human endothelial cells, a process that can overcome the downregulation induced by TNF-α [81]. The association of these receptors with caveolae could represent another means of regulating protein C activation [82]. As mentioned previously, the distribution of thrombomodulin and EPCR in specific vessel types also has implications for the differential regulation of coagulation.

Thrombin has divergent effects on the APC pathway. In vitro studies with human endothelial cells have shown that thrombin binding induces endocytosis of the thrombin-thrombomodulin complex, which could represent a mechanism for thrombin clearance and degradation as well as downregulation of protein C activation [83]. Conversely, and likely via independent receptors, thrombin can induce an increase in thrombomodulin transcription [84]. Although thrombin might be associated with endothelial shedding of EPCR, it also increases EPCR transcription in animal models [69, 85]. While difficult to assess in vivo, these results suggest that a surge of thrombin, as seen after injury, might lead to significant increases in EPCR and thrombomodulin transcription and expression.

One area of interest, especially with regard to TIC, is the effect of hypoxemia on protein C activation. Earlier in vitro studies using bovine aortic endothelium showed that hypoxemia led to marked selective decreases in the expression of thrombomodulin over several hours, which were reversible with re-oxygenation [86]. Similar effects were found in human endothelium in vitro [87]. This work suggests that shock and its attendant hypoxemia would lead to an overall decrease in protein C activation. However, several clinical studies have demonstrated the opposite: ischemia leads to increased protein C activation in vivo. This effect has been established in pigs, where selective coronary artery occlusion leads to a rapid increase in protein C activation that facilitates recovery from ischemic insult [88], and in humans, where brain vasculature APC levels rise within seconds of carotid artery occlusion [89]. Another clinical study, in the setting of cardiopulmonary bypass, demonstrated significant increases in APC levels in the minutes following aortic unclamping, with the degree of APC increase correlating with improvements in postoperative cardiovascular function [90].

Inactivation of protein C is mediated by protein C inhibitor (PCI), a member of the family of serine protease inhibitors (serpins) [91]. Like other serpins, PCI forms a covalent bond with its target, leading to APC degradation and a conformational change of the inhibitor [92]. Other known inhibitors of APC include α1-antitrypsin and α2-macroglobulin; in concert with PCI, these inhibitors facilitate the relatively long half-life of APC (approximately 20 min) [93, 94].

Anticoagulant Function of Activated Protein C

Inactivation of Coagulation Factors

Once activated and released from EPCR, APC achieves its primary anticoagulant effect by proteolytic inactivation of coagulation factors Va and VIIIa. This activity is dependent upon binding of the APC Gla domain to negatively charged phospholipid membranes [95]. Given that factor Va enhances the activity of the prothrombinase complex greater than 10,000-fold, APC-mediated inactivation of Va effectively ceases thrombin production [96, 97]. Proteolytic cleavage of Va at Arg506 is the more rapid reaction but only results in partial inactivation; cleavage at Arg306 leads to complete inactivation [10, 98]. As described previously, protein S serves as a potent cofactor in this cleavage, optimizing APC orientation for binding to the phospholipid membrane and the targeted substrate; protein S potentiates the more definitive Arg306 cleavage up to 20-fold [52, 53]. Overall, APC shuts down Va activity, preventing formation of thrombin via the extrinsic pathway and severely delaying primary clot formation.

Inactivation of factor VIIIa resembles that of Va, with APC-induced cleavage occurring at Arg336 and Arg562 [99]. Cleavage at either site induces functional inactivation [54]. Both protein S and inactivated factor V serve as cofactors in the inactivation of VIIIa, which has a relatively short half-life regardless of inactivation but may be protected by binding to other factors [100]. Activated factor VIIIa enhances formation of activated factor Xa via the intrinsic pathway up to 200,000-fold [101]. Thus, APC downregulates rapid thrombin formation via the extrinsic pathway through cleavage of factor Va as well as secondary thrombin formation via the intrinsic pathway through cleavage of factor VIIIa [97].

Fibrinolysis

In addition to its direct anticoagulant effects, APC has been associated with increased fibrinolysis; however, this relationship has recently become less clear. Early published characterizations of "autoprothrombin IIa" demonstrated induction of fibrinolysis on thromboelastography in both canine and human blood in vitro; an in vivo canine model of APC infusion yielded similar results [6]. These investigators suspected an indirect effect on plasminogen, which was confirmed in subsequent studies that identified a secondary-messenger mechanism by which APC produced increases in plasminogen activator [102, 103]. The effect of APC on decreasing plasminogen activator inhibitor 1 (PAI-1) activity was shown in whole-blood clot lysis assays [104, 105] and studies of cultured endothelium [106, 107]. The relevance of this mechanism was questioned when it could not be replicated in the primate setting [108] and proceeded slowly in a purified system with human proteins. In 2001, Rezaie showed that the glycoprotein vitronectin acts as a stabilizing cofactor to accelerate the reactivity of PAI-1 with APC in humans [109]; based on these findings, it appears that in vivo APC inactivates PAI-1, a serpin, in a reaction potentiated by vitronectin, causing de-inhibition of fibrinolysis.

Thrombin-activatable fibrinolysis inhibitor (TAFI), another mediator of fibrinolysis, is also linked to the APC pathway. Its major physiologic activator is the thrombin-thrombomodulin complex [110]. A carboxypeptidase enzyme, activated TAFI inhibits fibrinolysis by removing lysine residues from fibrin, preventing the activation of plasminogen [111, 112]. Before Rezaie demonstrated the role of vitronectin in potentiating the interaction between APC and PAI-1, TAFI was identified as a possible means by which APC could promote fibrinolysis: by its anticoagulant effect, APC reduces thrombin formation, limiting the key substrate that activates TAFI and leading to reduced inhibition of fibrinolysis [113, 114]. However, this mechanism is unclear because both protein C and TAFI are substrates for thrombomodulin and could compromise the activation of each other through competition. Thrombomodulin-dependent TAFI activation and fibrinolysis inhibition have been shown to occur in the presence of protein C [115]. The concentration of thrombomodulin has been proposed to regulate the counteracting effects of APC and TAFI on fibrinolysis, perhaps in a tissue-specific pattern based on thrombomodulin distribution within the vasculature [116]. Substrate preference of thrombomodulin might also be regulated by platelet factor 4, which when released from activated platelets promotes protein C activation and inhibits TAFI activation, leading to a profibrinolytic state [117]. Although these studies suggest a role for APC in fibrinolysis, the data in trauma patients is conflicting, as discussed later.

Cytoprotective Effects of Activated Protein C

APC's anti-inflammatory effects were initially attributed to decreased production of proinflammatory thrombin. Thrombin has been shown to exert inflammatory actions on cells, primarily via GTP-binding protein-coupled protease-activated receptors (PARs) [118], and APC likely plays an indirect role in mitigating this activity through its anticoagulation pathway. Early awareness of a direct relationship between APC and inflammation was postulated when thrombin infusion led to increased survival in a canine sepsis model and was associated with anticoagulation and enhanced fibrinolytic activity [119]. These findings prompted directed investigation of APC's effects in a primate model of sepsis, in which APC prevented the lethal effects of high-dose *Escherichia coli* infusion in baboons and APC blockade exacerbated the effects of sublethal *E. coli* challenge [120]. These studies, conducted in the late 1980s, paved the way for randomized controlled trials of recombinant human APC therapy for sepsis two decades later.

Based on the growing body of animal data indicating an APC-induced anti-inflammatory effect beyond attenuation of thrombin production, Joyce and colleagues performed transcriptional profiling that showed recombinant human

A L G r a w a n y

APC modulates human endothelial cell gene expression, with resulting inhibition of major apoptotic and inflammatory pathways [121]. Shortly thereafter, APC was shown to signal cells directly through the PAR-1 receptor, an interaction dependent on APC-to-EPCR binding and affecting gene expression of intracellular regulators of apoptosis and inflammation [12]. Since that discovery, a series of experiments have identified a pleiotropic array of candidate receptors and pathways for APC signaling on multiple cell types, including endothelium and immune cells [122].

At this time, the paradigmatic model for APC's cytoprotective effects focuses on EPCR and PAR-1. After activation, APC bound to EPCR induces specific noncanonical N-terminal cleavage and activation of PAR-1, resulting in a series of intracellular processes distinct from those induced by thrombin-PAR-1 binding [123, 124]. These effects depend to some extent on the cell type and include the regulation of gene expression via suppression of nuclear transcription factor activity [121]. In endothelial cells, PAR-1 signaling inhibits vascular adhesion molecule expression and release of proinflammatory molecules [125]. Endothelial barrier function is enhanced in vitro by APC, an effect dependent upon PAR-1 signaling and mediated via the sphingosine-kinase pathway and sphingosine 1-phosphate receptor [126]. In leukocytes, APC prevents adhesion molecule expression and reduces the release of pro-inflammatory mediators like TNF-α, potentially attenuating the cytokine storm that characterizes sepsis and post-injury inflammation [127, 128]. APC has been shown to inhibit apoptosis in an EPCR- and PAR-1-dependent manner [129], and in hypoxic-stress states APC downregulates proapoptotic proteins like p53 and Bax in human brain endothelium [130]. While many of these mechanisms have been delineated in vitro, they are supported by ongoing animal experiments, especially those that employ genetic knockouts, domain-specific targeted mutants of APC, and selective PAR-1 agonists [131, 132]. These studies have taken place while large-scale human trials of recombinant APC for therapeutic use in sepsis have been conducted, as discussed later.

Independent of the PAR-1 cell-signaling pathway, APC attenuates inflammation in an indirect fashion via inactivation of extracellular histones. APC cleaves histones and reduces their cytotoxic effects in vitro, and in a mouse model, APC infused with lethal doses of histones prevented death [133].

Clinical Relevance of the Protein C Pathway

Protein C was discovered and characterized in the laboratory before any awareness of its clinical significance. In more recent years, studies have shown that genetic deficiencies in the APC pathway from protein C [134] to thrombomodulin [34] to EPCR [135] can lead to early thrombosis, hemorrhage, and embryonic or perinatal lethality. These findings underscore the essential role APC plays in maintaining hemostatic balance.

The first clinical syndrome related to protein C was reported in 1981, when Griffin and colleagues identified a 22-year-old man with multiple thromboembolic complications and a family history of recurrent thrombotic disease. In the patient and two affected family members, depressed levels of protein C were identified [136]. Two years later, a Dutch study described congenital protein C deficiency in three unrelated families with high rates of thrombophlebitis and, through pedigree analysis, posited that the disorder was transmitted in an autosomal-dominant fashion with incomplete penetrance [137]. Shortly thereafter, independent investigators reported increased thrombosis in patients with hereditary protein S deficiency [138, 139]. Since these initial discoveries, many genetic mutations have been identified that lead to quantitative and qualitative protein C or S deficiencies; the majority of identified patients are heterozygous and manifest higher rates of venous thrombosis [140]. Homozygous protein C deficiency occurs more rarely, likely due to fetal demise, and manifests as neonatal purpura fulminans, a highly morbid condition that can be treated with protein

C concentrate [141, 142]. Similar pathophysiology is observed in acquired protein C deficiency, the classic example being the occurrence of skin necrosis following the initiation of warfarin. In this situation, the relatively short half-life of protein C leads to predominance of longer half-life vitamin K-dependent procoagulant factors, with resulting thrombosis in the microvasculature [143].

Disappointed by the lack of identifiable protein C or S deficiency in many patients with apparently familial thrombosis syndromes, Swedish researchers sought to find alternative genetic mechanisms and in 1993 identified a heritable resistance to APC [144]. A simultaneous Dutch study arrived at similar conclusions [145]. This phenotype was found to be more prevalent than any previously known deficiencies and is now considered the most common cause of inherited thrombophilia [146]. The following year, three groups, including University Hospital in Leiden, showed that APC resistance is characterized by a single-nucleotide substitution mutation in Factor V, in which the arginine at APC-cleavage site Arg506 is replaced with glutamine, preventing the inactivation of factor Va by APC [147]. Factor V Leiden remains the best characterized heritable thrombophilia and is found in heterozygous form in up to 5% of Caucasians. It is thought to have arisen from a single ancestor 21,000–34,000 years ago and persisted due to the relative benefits of hypercoagulability (namely, decreased hemorrhagic death) in the premodern era [148].

Since the turn of the twenty-first century, the anti-inflammatory actions of APC have garnered interest in human sepsis trials. Following the protective effects of APC in baboon sepsis studies, a large international prospective multicenter trial was conducted to assess the safety and efficacy of activated drotrecogin alfa (recombinant human APC, rhAPC) in the treatment of sepsis in humans. This randomized, placebo-controlled, phase 3 study was discontinued early when a mortality benefit was found in rhAPC-treated patients: these patients had a reduction in relative risk of death at 28 days of 19%, with an absolute reduction of 6% [149]. This finding represented the first successful non-antimicrobial pharmacologic therapy for severe sepsis, and its mortality benefit across subgroups was hailed as a breakthrough in critical care medicine [150]. However, subsequent investigations in less severely ill and pediatric populations failed to show a similar benefit and raised questions about the risk of bleeding complications [151, 152]. A follow-up multicenter trial failed to show any benefit to rhAPC treatment, and as a result rhAPC was withdrawn by the manufacturer [153]. The comparability of this study to the original trial has been questioned, along with its statistical power in the setting of a low mortality rate; subsequent meta-analyses have suggested that there might be a significant therapeutic benefit to rhAPC [154, 155].

Spurred by the initial success of rhAPC in sepsis, several animal and human studies have been conducted to assess further therapeutic applications. Animal studies indicated that APC administration might attenuate lung injury, but these results were not replicated in a human multicenter randomized controlled trial [156, 157]. Recombinant forms of APC have shown benefits in preclinical models of ischemia and reperfusion injury of the heart, liver, and kidney; diabetic nephropathy; and radiation toxicity [158–161]. Anti-inflammatory effects combined with the promotion of angiogenesis make APC a promising therapeutic for chronic wound healing [162, 163]. Multiple animal studies have indicated a neuroprotective role for APC, especially in the setting of ischemia [130, 164–166]. These findings have prompted human studies of a cytoprotective-selective APC variant in the treatment of ischemic stroke, which are currently in Phase II trials [167]. The ability to deliver domain-specific agents at higher doses than those used in prior rhAPC trials (since the risk of bleeding can be eliminated by inactivation of the anticoagulant function) could prompt re-evaluation of therapeutic applications in sepsis. Recombinant thrombomodulin is also being studied in the management of sepsis-associated coagulopathy but thus far has not been found to have a mortality benefit [168].

Activated Protein C and Trauma-Induced Coagulopathy

Although post-traumatic coagulation abnormalities had been classically attributed to coagulation factor consumption or iatrogenic interventions (the "vicious triad" of hemodilution, hypothermia, and acidosis), in 2003 two independent cohort studies reported that roughly 25% of trauma patients present with coagulopathy on admission prior to resuscitation [169, 170]. Those with impaired coagulation had increased transfusion requirements, increased rates of organ dysfunction, and a four-fold increase in mortality. Coagulopathy corresponded with injury severity, but its effect on mortality was independent of Injury Severity Score (ISS). Unable to determine the causal mechanism of this newly identified "endogenous" coagulopathy, the authors posited that it might "be an indicator of loss of regulation of the local inflammatory response" [170].

Our group studied 208 trauma patients and hypothesized that the combination of tissue injury and hypoperfusion might lead to a pathologic activation of the APC pathway. Measuring tissue hypoperfusion by base deficit, we found that coagulopathy occurred only in the presence of significant base deficit (>6 mEq/L) regardless of total thrombin formed (prothrombin fragments 1 + 2 levels) (Fig. 10.2) [171]. With normal fibrinogen in all patients, this finding argued against coagulation factor consumption as the underlying etiology, as would be seen in disseminated intravascular coagulation. Increased base deficit was also associated with decreased zymogenic protein C and increased soluble thrombomodulin; while protein C also decreased with thrombin formation, it only did so in the presence

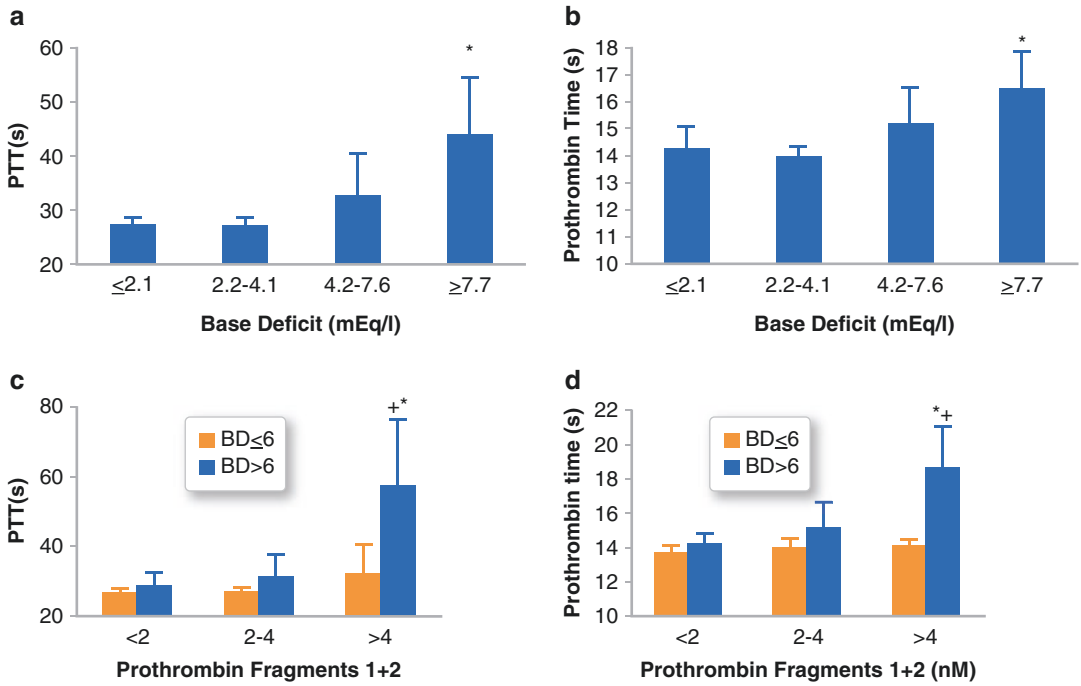


Fig. 10.2 Effects of tissue hypoperfusion on coagulation. Partial thromboplastin time (PTT, **a**) and prothrombin time (**b**) are prolonged with increasing base deficit (BD). PTT (**c**) and prothrombin time (**d**) are prolonged only in the presence of elevated BD. When BD is ≤6 (orange bars), increasing thrombin generation (represented by prothrombin fragments 1 and 2) has no effect on PTT or prothrombin time; when >6 (blue bars), prolongation of coagulation parameters is seen with increasing thrombin generation. *, $p < 0.05$. (Modified from Brohi et al. [171])

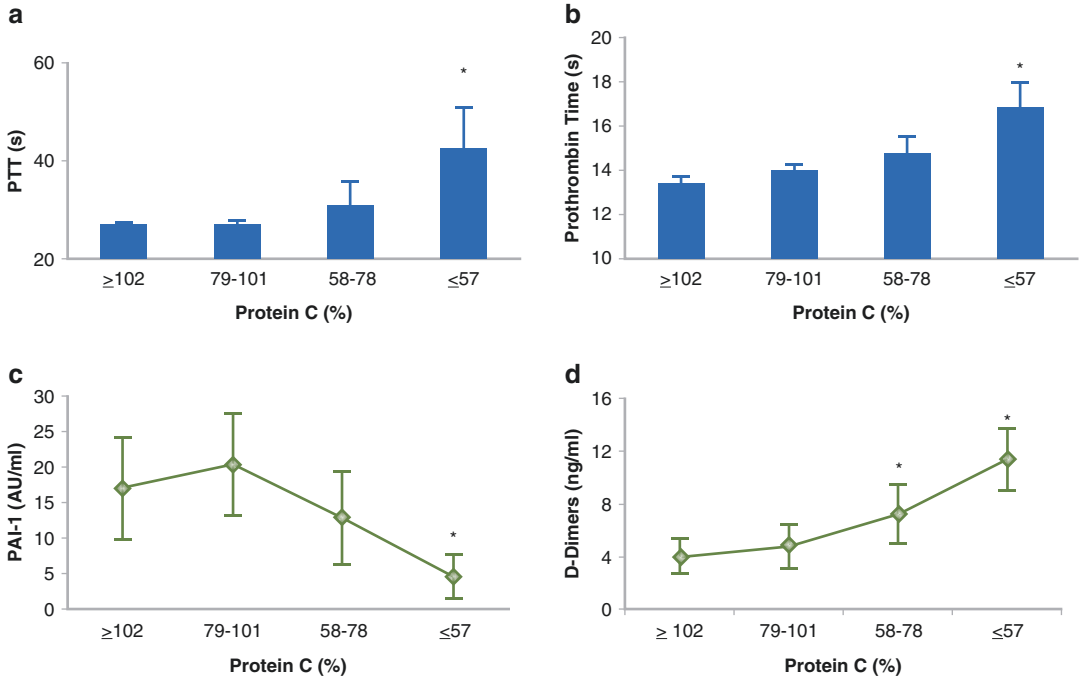


Fig. 10.3 Indirect evidence for protein C activation. Partial thromboplastin time (PTT, **a**) and prothrombin time (**b**) are prolonged as protein C decreases. Plasminogen activator inhibitor-1 (PAI-1) is reduced (**c**) and D-dimers are elevated (**d**) as protein C decreases. *, $p < 0.05$. (Modified from Brohi et al. [171])

of increased base deficit. Decreased protein C correlated with prolonged PT and PTT, decreased PAI-1 activity, and increased D-dimer, indicating a hypocoagulable and fibrinolytic state (Fig. 10.3). Transfusion requirements, complications, and mortality were associated with decreased protein C zymogen and increased soluble thrombomodulin.

This first study linking APC and TIC was not without limitations, primary the lack of direct measurement of the activated form of protein C. Likewise, the significance of soluble thrombomodulin remains unknown and might not be a direct assay of overall thrombomodulin activity. The data was also collected at a single time point, upon admission. Most importantly, the findings represented a compelling correlation but fell short of definitive causal evidence. Nevertheless, the hypothesis and results generated considerable discourse, especially regarding the therapeutic implication that reversal of shock might be more critical than coagulation factor repletion in trauma resuscitation [172].

In a clinical study published the following year, increases in soluble thrombomodulin correlated with decreased utilization of fibrinogen, decreased zymogenic protein C, and increased TAFI, indicating that soluble thrombomodulin levels might correlate with increased overall activity [173]. In an attempt to clarify the mechanisms of fibrinolysis, tPA was shown to be increased in the presence of decreased PAI-1 levels, which correlated with D-dimer, whereas TAFI had no significant correlation with D-dimer. These findings suggested that the primary effect on fibrinolysis was mediated via decreased PAI-1 (and thus increased tPA and plasmin) rather than protein C competing with TAFI for thrombomodulin binding sites (and thus decreasing overall TAFI activity).

Three years later, in a study of 203 severely injured patients, the definitive evidence of protein C activation was established [174]. APC levels were measured using an enzyme-capture assay and correlated with decreased zymogen protein C. Patients with severe injury and shock had

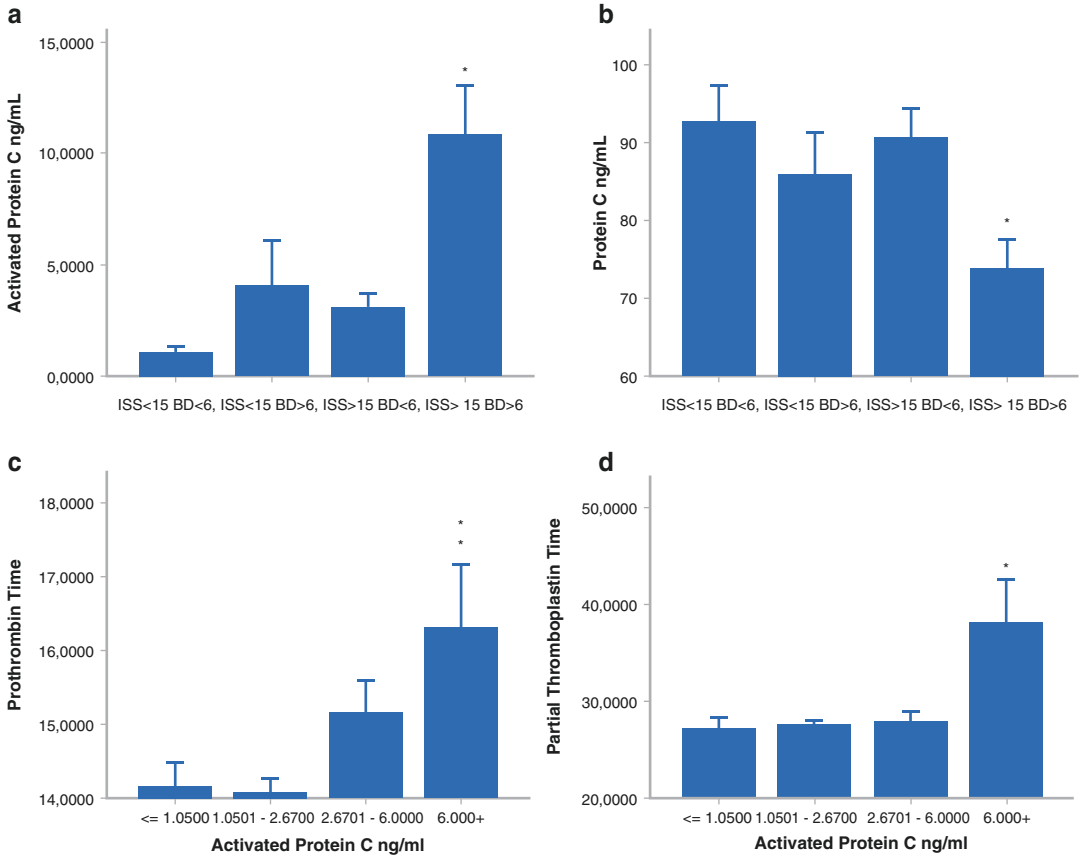


Fig. 10.4 Tissue injury and shock result in systemic activation of the protein C pathway. Patients were divided into four groups based on Injury Severity Score (ISS) and base deficit (BD): minimal injury without shock (ISS < 15, BD < 6); minimal injury with shock (ISS < 15, BD > 6); severe injury without shock (ISS > 15, BD < 6); and severe injury with shock (ISS > 15, BD > 6). Patients with severe injury and shock had elevated levels of activated protein C (**a**) and reduced levels of protein C (**b**). Patients with the highest quartile of activated protein C had prolonged prothrombin time (**c**) and partial thromboplastin time (**d**). *, $p < 0.05$. (Modified from Cohen et al. [174])

elevated levels of APC (Fig. 10.4), and APC correlated with the degree of coagulopathy in linear regression, wherein a log increase in APC resulted in a 2.4-s increase in PT. APC was inversely related to levels of factors Va and VIIIa and directly related to increases in tPA and D-dimer. With regard to outcomes, the degree of protein C activation (by APC:PC ratio on admission) correlated with increased odds of ventilator-associated pneumonia (VAP), lung injury, and multi-organ failure, as well as 2.1-fold increased odds of mortality. Analysis of longitudinal protein C levels following admission showed that increased APC with failure to recover normal zymogen levels correlated with increased risk of VAP.

These results were obtained using prospectively collected data from a single institution. To investigate the mechanistic drivers of TIC in a larger, multicenter context, an analysis was conducted using the Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) study. Among 1200 patients from ten centers, shock and base deficit predicted admission coagulopathy [175]. Analysis of coagulation factors identified protein C activation (decreased protein C zymogen, increased APC) and coagulation factor depletion, especially V and VIII, as predictive of coagulopathy.

Since results from human studies remain by design correlative and observational, a traumatic

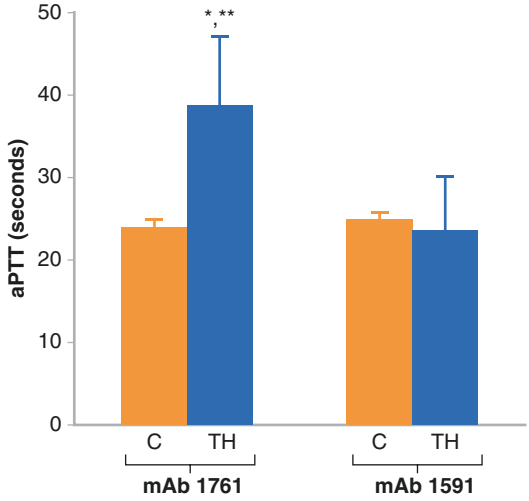


Fig. 10.5 Inhibition of the anticoagulant function of protein C prevents the development of acute traumatic coagulopathy in mice. Mice were treated with a monoclonal antibody that inhibits the anticoagulant function of protein C (mAb 1591) or an isotype control (mAb 1761). After 10 min, the mice underwent traumatic hemorrhage (TH). Activated partial thromboplastin time (aPTT) was measured after 60 min of hemorrhagic shock. *, $p < 0.05$. (Modified from Chesebro et al. [176])

hemorrhage model was developed in mice to investigate these mechanisms. In this model, tissue injury combined with hypoperfusion led to reproducible coagulopathy. A directed antibody, mAb 1591, was used to block the anticoagulant domain of APC and prevented traumatic coagulopathy [176] (Fig. 10.5). This finding provided mechanistic evidence that APC can mediate TIC. Interestingly, all mice pretreated with an antibody that blocks the anticoagulant and cytoprotective domains of APC (mAb 1609) died within 45 minutes of shock initiation and had diffuse pulmonary arteriolar thrombosis accompanied by perivascular and alveolar hemorrhage. This result underscored the key role APC plays in mitigating systemic inflammation. In a rat model of penetrating traumatic shock, thromboelastography indicated that TIC was due to impaired thrombin formation, favoring APC's inactivation of Va and VIIIa as the underlying mechanism [177].

Based on this mechanistic data and the human clinical results, our group posited that TIC represents an evolved but maladaptive response to severe injury. In the setting of severe trauma and tissue hypoperfusion, an excess of protective anti-inflammatory APC is released in an attempt to prevent local microvascular thrombosis and mitigate cellular dysfunction (Fig. 10.6). This view is supported by data from a trauma cohort demonstrating that poor outcomes associated with increased levels of inflammatory histones are abrogated by simultaneous increases in endogenous APC, implying a protective effect of APC in the setting of widespread inflammation [178]. According to this hypothesis, APC may be activated as part of an innate response to inflammatory injury, resulting in an "inadvertent" anticoagulant effect and leading to the development of a clinically significant coagulopathy. The subsequent depletion of the overactivated protein C system exposes the individual to eventual organ injury and infection.

Several questions remain regarding this hypothesis. One mechanistic element relates to the driver of protein C activation in the setting of traumatic shock. While thrombin is readily available after tissue injury, upregulation of thrombomodulin in hypoperfused states remains debated. As discussed previously, in vitro studies have indicated that hypoxemia might decrease thrombomodulin expression [86, 87], but multiple studies have demonstrated that ischemia leads to increased protein C activation in vivo [88–90].

Another area of interest is the upstream drivers of APC-induced TIC. Activation of protein C is initiated by thrombin, but the mechanisms of thrombin production in trauma have not been characterized. While the roles of tissue factor and the extrinsic pathway in hemostasis have been long established [179], recent findings have suggested that alternative inflammatory mediators could drive changes in coagulation dynamics, possibly via the intrinsic pathway [180–182]. To elucidate which pathway predominates in coagulation activation after trauma, targeted antibody blockades were employed in the previously described murine model of traumatic shock [183]. These data indicate that coagulation activation following traumatic hemorrhage is driven primarily by the (extrinsic) tissue factor pathway

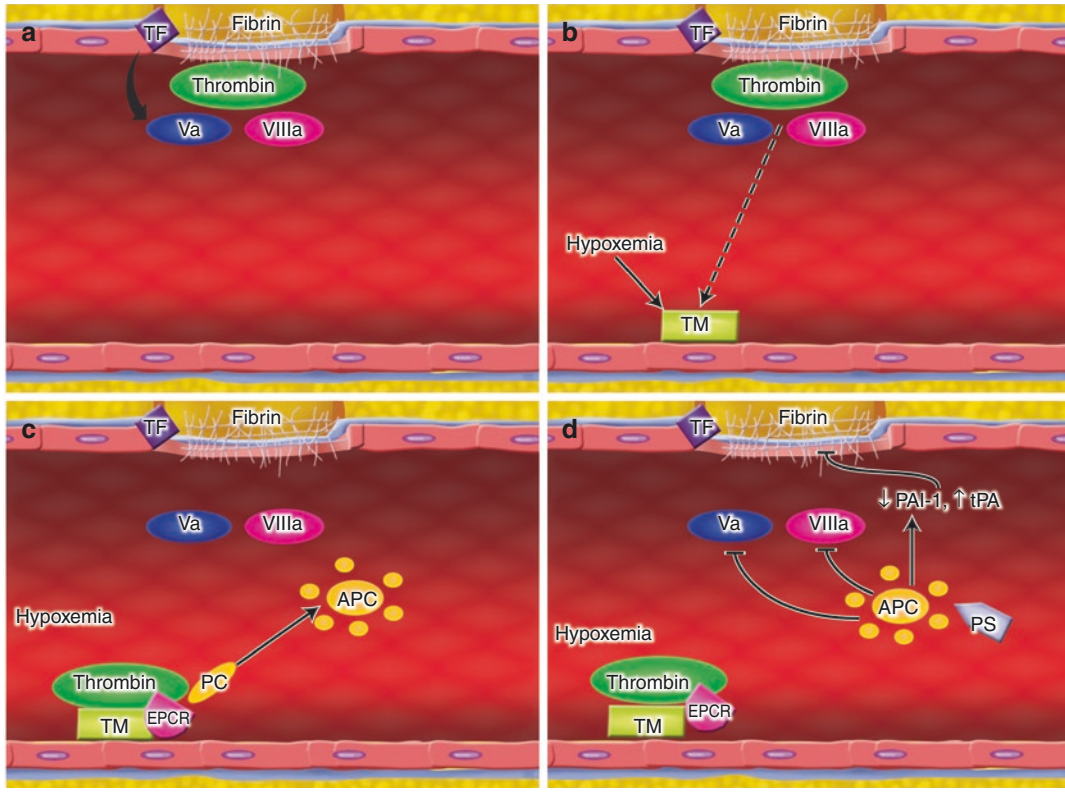


Fig. 10.6 Activated protein C pathway in trauma-induced coagulopathy. (**a**) Tissue injury exposes tissue factor (TF), which drives thrombin formation via the coagulation cascade, resulting in production of fibrin clot. (**b**) Hypoxemia leads to increased expression of endothelial thrombomodulin (TM), which diverts thrombin from clot formation. (**c**) Thrombin bound to TM activates zymogenic protein C (PC) in a reaction potentiated by endothelial protein C receptor (EPCR). (**d**) Assisted by protein S (PS), activated protein C (APC) inactivates Va and VIIIa by proteolytic cleavage and disinhibits fibrinolysis by inhibiting PAI-1, leading to a hypocoagulable state

and that the resulting increase in thrombin production is responsible for the activation of protein C seen in TIC.

An in vitro study involved adding increasing levels of APC to healthy subjects' whole blood and concluded that the relatively APC-resistant population of Va on platelets effectively negated any hypocoagulable effects from APC at or above reported in vivo concentrations [184]. Although the authors surmised that these findings precluded the role of APC in TIC, the study did not incorporate an endothelial element, nor did it acknowledge the fact that tissue-specific concentrations of APC in injured areas may differ from those measured at a site of remote venipuncture [185]. These conclusions contradicted thromboelastometry data from healthy subjects collected a decade before to assess the potential coagulation effects of rhAPC administration; that study demonstrated a significant increase in clotting time at relatively low doses of rhAPC [186]. Another *in vitro* study showed a dose-dependent effect of APC on coagulation dynamics, with hypocoagulability (but albeit reduced fibrinolysis) by thromboelastometry at increasing doses [187]. While these studies might provide insights into the mechanisms of TIC, they are limited by their ex vivo context.

Another area that remains unclear is the role of APC in regulating fibrinolysis. Although preclinical work has suggested that APC can drive fibrinolysis, the data on this topic in the trauma literature is mixed. As discussed previously, in trauma patient plasma, reduced zymogenic pro-

tein C and elevated APC have been associated with increased fibrinolytic activity [171, 174, 188, 189]. These findings are supported by a recent animal model of traumatic hemorrhage, in which fibrinolysis and fibrinogen depletion were attenuated in mice with a genetic thrombomodulin mutation and therefore reduced ability to activate protein C [189]. However, when APC has been added to healthy human whole blood, fibrinolysis was reduced on thromboelastometry [187]. Moreover, PAI-1 is now thought to form a complex with tPA instead of being degraded after trauma, conflicting with the hypothesis that APC disinhibits fibrinolysis by neutralizing PAI-1 [190]. The regulation of fibrinolysis after injury, including the role of APC in that process, remains an area of continuous investigation.

Throughout this discussion, attention has been focused on the APC pathway as a potential causal agent in TIC; however, this approach should not be misunderstood as an attempt to characterize APC as the only, or even paramount, driver of TIC. TIC represents a complicated and likely heterogeneous pathophysiology, incorporating multiple pathways of inflammation, coagulation, and fibrinolysis [191]. Application of more sophisticated statistical methods, including principle components analysis, to understanding TIC dynamics in clinical populations indicates that APC-induced coagulopathy likely represents one phenotype among many [188].

Future Directions and Therapeutic Implications

Based on the results of mechanistic murine studies, one approach might be to develop a human antibody similar to murine mAb 1591, which blocks APC's anticoagulant domain and prevents coagulopathy in the setting of traumatic shock [176]. While blocking APC anticoagulation in trauma might attenuate coagulation abnormalities, the potentially prothrombotic complications would be concerning, and extensive preclinical testing would be required. Also, determining the appropriate patients for therapy and defining the optimal time after injury for administration are significant challenges.

One promising area of APC investigation is domain-specific variants. A more thorough understanding of APC's structure and related functional implications has led to the development of engineered APC that is selective for specific functions. As noted, following the concerns of bleeding complications and limited clinical benefit related to rhAPC, variants with diminished anticoagulant but intact cytoprotective function have been engineered [192]. Site-specific mutagenesis has preserved EPCR binding and PAR-1 cleavage while nearly eliminating proteolytic coagulation factor inactivation [193]. While this development has obvious appeal in the treatment of sepsis and related inflammatory conditions, including the ability to deliver much higher doses than tolerated in the original rhAPC trials, such variants might be able to provide a potent therapeutic option in the setting of trauma. By administering a cytoprotective-selective variant following significant injury with attendant shock, a system depleted of APC could be buffered against inflammatory sequelae such as nosocomial infection and organ damage. Again, the optimal patient selection and timing of such therapeutics would need to be studied carefully. Lastly, the use of variants can be complemented by genetic knockouts and overexpressors in animal models; these studies will enrich our growing understanding of the complex APC pathway dynamics in the setting of traumatic shock.

Though the initial clinical and mechanistic characterizations of APC in TIC have been reviewed here, the translation of such knowledge into potential interventional approaches remains in nascent stages. The promise of incorporating this increased awareness of APC's role into an integrated understanding of TIC to treat critically injured patients represents a research priority now and for the foreseeable future.

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Fibrinolysis Dysregulation Following Trauma

11

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Historical Perspective

The association between trauma, fibrinolysis, and death has been recognized for over 200 years. One of the first described coagulation changes during hemorrhage was from Hewson in 1772, who stated in sheep "the blood which issued last coagulated first" [1]. John Hunter, from his wartime experiences at the end of the 1790s, observed incoagulability from victims of lethal gunshot wounds [2]. Nearly a century later it was discovered that liquid whole blood could dissolve formed clots and the term "fibrinolysis" was proposed. Blood removed from corpses from sudden death proved to be a useful source of blood for transfusions, as their blood would initially clot, but then reliquify and, thus, not require an anticoagulant. Macfarlane et al. [2] concluded that the commonality of these early observations was rapid death resulting in reversal of clot formation. This was experimentally confirmed by Cannon and Gray in 1914 [3] in a dog model of hemorrhagic shock, in which it was appreciated that during progressive hemorrhage, clotting time was reduced. This reduction in clotting time was ascribed to a protective mechanism to stop bleeding, but at the cost of pathologic hypercoagulability in the microvascular system resulting in irreversible shock, as described by Crowell and Read in 1955 [4]. Turpini and Stanfanini [5] several years later demonstrated experimentally that fibrinolysis was activated during hemorrhagic shock to counterbalance shortened clotting time. This observation was further strengthened in an animal model of hemorrhagic shock in which heparin pretreatment markedly reduce mortality. This was subsequently validated by Hardaway et al. who documented that pretreating animals with heparin eliminated microvascular clots following hemorrhagic shock [6]. Hardaway's group subsequently found that a profibrinolytic would also reduce mortality if given after hemorrhagic shock [7], further supporting the role of microvascular thrombi in irreversible shock. Whitaker and McKay around the same time showed that epinephrine infusions in animals would similarly result in intravascular fibrin deposition, which was prevented with heparin and exacerbated with an antifibrinolytic [8].

The term disseminated intravascular coagulation (DIC) [9] emerged during this time and implicated multiple visceral thromboses as the cause of shock. Fibrinolysis was proposed to be a physiologic process to counterbalance these thromboses, and when fibrinolysis was inhibited after hemorrhage, it resulted in irreversible shock

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and death [10]. Taking organ biopsies in a patient that is bleeding with organ failure is not a benign intervention and clinically not feasible in most situations to determine if a patient is in DIC. This resulted in laboratory measurements to estimate what is occurring at the microvascular level to define DIC employing a score including fibrinogen level, platelet count, fibrin degradation product, and prothrombin time [11] without histologic proof. Extrapolation of these laboratory measurements has led to the misinterpretation of defining DIC as pathologic "in vivo" activation of coagulation [12]. This may be true in certain clinical scenarios such as malignancy [13] and sepsis [14] when pathogen or abnormal tissue creates a prothrombotic surface and causes consumptive coagulopathy. However, in trauma, in vivo activation of coagulation is a survival mechanism to prevent uncontrolled bleeding and thus is a physiologic response to injury that is different in etiology to other disease states such as malignancy, sepsis, and pregnancy. Regardless of the limitations in defining DIC, it has always been perceived to be the result of a secondary event due to an underlying primary disease process [15]. Therefore, treating the initiating trauma-induced coagulopathy to return the patient to homeostasis is the goal, and fibrinolysis management is a critical component.

This balance of coagulation was emphasized in Stafford's review on fibrinolysis and hemostasis in 1964 [15] and established a logical explanation for how fibrinolysis in both extremes could become pathologic: "a general assumption has been developed that clotting is not episodic but a continuous process which is normally never allowed to progress to a physical end point." Pathologic hyperfibrinolysis was defined by Starzl et al. using thrombelastography (TEG) during the early operative phase of liver transplant [16], while Hardaway in 1965 described fibrinolysis shutdown following trauma and prolonged hemorrhagic shock [17]. Recently, it has been recognized that trauma patients can present with a spectrum of fibrinolysis within 12 hours of injury [18] (Fig. 11.1). Viscoelastic hemostatic assays (VHA) to quantify the two pathologic ends of the fibrinolysis spectrum (excessive clot degradation, hyperfibrinolysis, and impaired clot degradation, fibrinolysis shutdown) have consistently demonstrated that moderate levels of fibrinolysis (physiologic) measured have the lowest mortality compared to hyperfibrinolysis and fibrinolysis shutdown [18–25].

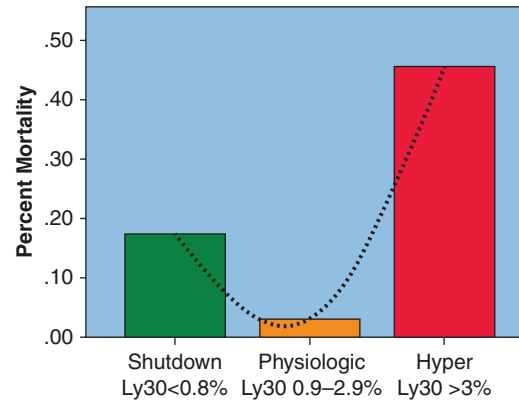


Fig. 11.1 Thromboelastic measurements of 200 critically injured patients (injury severity score > 15) identified the spectrum of fibrinolysis. Patients with a moderate (physiologic) level of fibrinolysis had the lowest mortality. Those patients with fibrinolysis above this range (hyperfibrinolysis) had nearly a 50% mortality, while those patients with impaired fibrinolysis (shutdown) had nearly a fourfold rate mortality compared to the physiologic range. These data implicate a protective role of fibrinolysis following severe injury

Challenges of Measuring Fibrinolysis

Systemic Versus Local Fibrinolysis

The regional distribution of specialized endothelial cells contributing to the fibrinolytic system is reflective of the importance of localization of this process [26]. The primary driver of intravascular fibrinolysis, tissue plasminogen activator (tPA), is released from precapillary arteriole and postvenular endothelial cells. This allows for a rapid response to prevent low-flow occlusion of small vessels. The body is continuously clearing fibrin deposition, and after trauma with extensive thrombin generation from tissue injury, this maintenance process is increased to maintain microvasculature patency distal to the injury site.

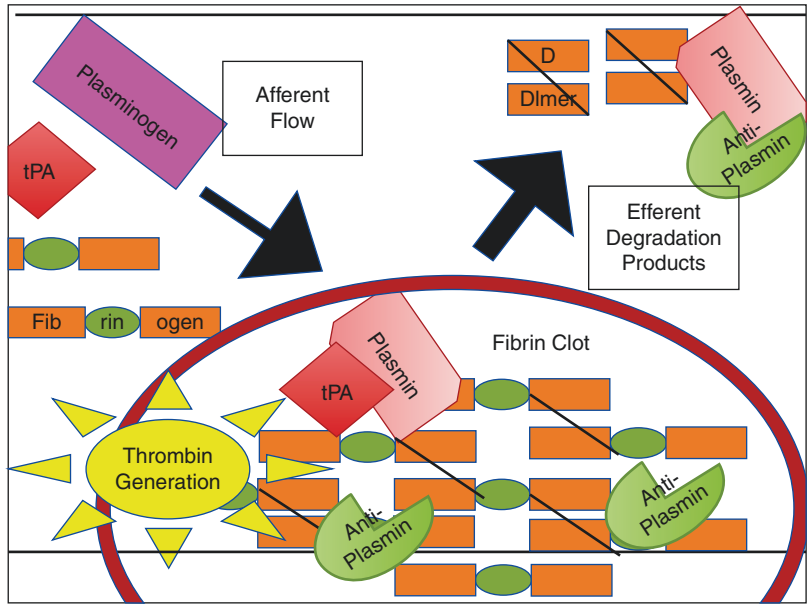


Fig. 11.2 Afferent flow to an area of injury provides fibrinogen which is acted upon by a local thrombin burst creating a fibrin seal which becomes cross-linked by factor XIII that also integrates antiplasmin proteins. Circulating tPA and plasminogen enter the clot and bind fibrin on site-specific lysine residues. This results in the cleavage of plasminogen into plasmin. Plasmin actively cleaves polymerized cross-linked fibrin and releases degradation products including D-dimer. As the clot degrades, antiplasmin proteins are uncovered and directly bind plasmin resulting in the formation of plasmin-antiplasmin complexes, which are released into systemic circulation

This explains why a D-dimer test is nonspecific. Local fibrinolysis will release fibrin degradation products systemically (Fig. 11.2). However, this does not necessarily correlate with an increase in systemic fibrinolytic activity (Fig. 11.3). Elevation of D-dimer can be a physiologic reaction to trauma, and correlation to systemic hyperfibrinolysis requires additional assays. Furthermore, the clearance of D-dimer exceeds 12 hours [27], and elevated levels of D-dimer can reflect prior activation of the fibrinolytic system but do not represent a patient's real-time systemic fibrinolytic activity.

Plasma Markers of Fibrinolysis Have Limitations

The limitations of the "gold standard" euglobulin lysis test (plasma based) have been discussed since 1962, with emphasis on the importance of developing whole blood assay to measure fibrinolysis that includes the important contributions of cellular components [28]. This is further emphasized by the lack of fibrinolysis reversal with certain antifibrinolytic medications due to these medications not precipitating out of plasma for this assay [29]. Perhaps the most important component of whole blood removed from the euglobulin lysis test is the platelet. Platelets provide a structural framework to resist fibrinolysis. During primary hemostasis, platelets aggregate and form adhesions between themselves and the surrounding environment including collagen, facilitated by Von Willebrand factor. This creates a unique microenvironment in which fibrin polymerization can accelerate on a phospholipid membrane for thrombin burst and regionalization of clotting components away from the circulating vascular system [30]. This area is further enriched with prothrombotic and antifibrinolytic proteins after platelet degranulation [31]. A low concentration of lysed platelets has been shown to reverse tPA-enriched whole blood fibrinolysis

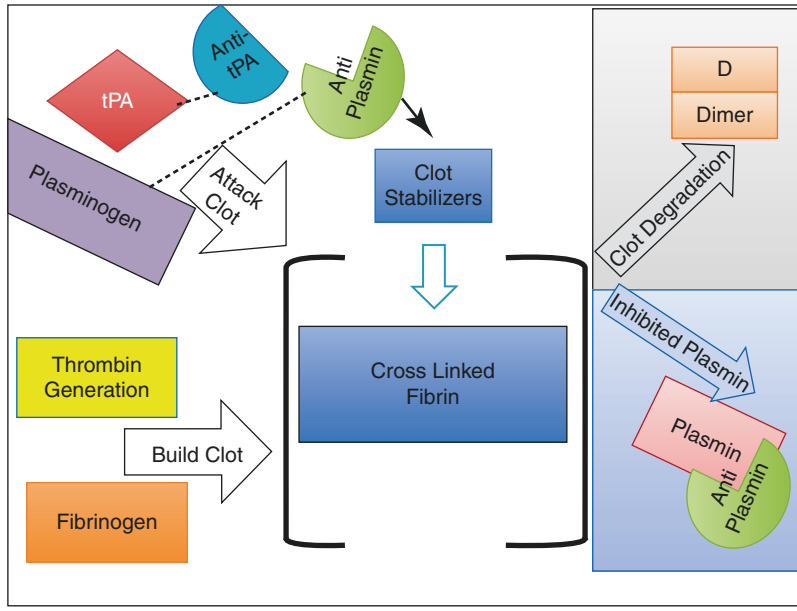


Fig. 11.3 Measurement of D-dimer and plasmin-antiplasmin (PAP) complexes is the result of the degradation of fibrin clot. Therefore, the systemic circulating levels of these plasma proteins are dependent on the patient's clot burden. The elevation of these products can be due to increased activity of the plasmin system, increased clot burden from extensive tissue injury or intravascular thrombosis, or decreased clearance of these products. They do not necessarily reflect the systemic activity of the fibrinolytic system in circulation. Microvascular beds can have elevated fibrinolytic activity in efforts to keep the vasculature patent increasing D-dimer and PAP levels, but not resulting in systemic hyperfibrinolysis. Acute pulmonary embolism is an example of an elevated D-dimer level but not systemic hyperfibrinolysis. An acute occlusion of the pulmonary vasculature results in a rapid release of local tPA to clear fibrin clot in the pulmonary vasculature and an elevation in D-dimer. Measurement of systemic fibrinolysis would be within a normal range as the circulating regulator proteins would buffer the local surge tPA released from the pulmonary system preventing systemic hyperfibrinolysis. In polysystem trauma patients, it is not a mystery why these protein levels have poor specificity for identifying the hyperfibrinolytic phenotype, as the body is actively building and degrading clot to optimize tissue perfusion. This only becomes pathologic when activity becomes unregulated and results in excessive clot degradation in areas of vascular injury resulting in refractory bleeding

[32]. The mechanism is perceived to occur from alpha-granule degranulation of the platelet in response to thrombin, which results in the release of plasminogen activator 1 (PAI-1), which can effectively inhibit tPA [11]. Because platelets play an important role in the regulation of fibrinolysis, non-whole blood assays are limited because they have eliminated important inhibitors of the fibrinolytic system.

Viscoelastic Assays and Fibrinolysis

ROTEM and TEG provide clinically useful measures of fibrinolysis (results within 1 hour: Fig. 11.4). The threshold between 3% and 15% LY30 (percent degradation of clot 30 minutes after reaching maximum amplitude) have been identified as an increased risk for massive transfusion and increased mortality [33–35]. The hyperfibrinolytic patient is relatively easy to identify with these assays, although the optimal activator and the optimal percent lysis threshold remain to be defined. Evaluating the other end of the spectrum of the fibrinolytic system, fibrinolysis shutdown, is more challenging. Appreciation of the two extremes of the fibrinolytic system in response to trauma and surgery dates back to the 1960s [36]. As previously mentioned, a spectrum of fibrinolysis is present in acutely injured

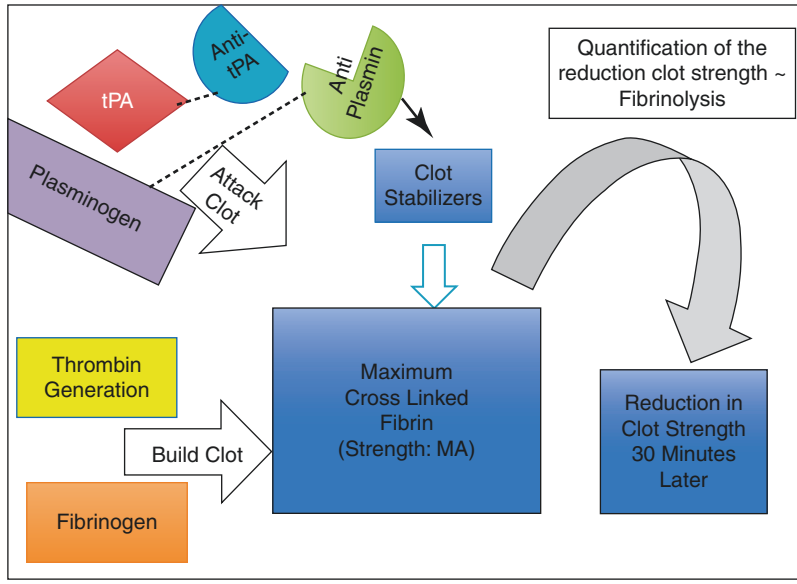


Fig. 11.4 Thrombelastography measures the progression of clot formation to maximum strength from the systemic blood of a patient in an in vitro environment. There are numerous contributing factors to clot strength including thrombin generation, platelet aggregation/degranulation, and clot stabilization such as factor XIII that are indirectly measured by this assay. In this in vitro environment, the clot will degrade after reaching maximum clot strength. The reduction in clot strength after 30 minutes represents clot weakening from fibrinolytic activity and to some degree platelet relaxation. This is dictated by the balance of pro- and antifibrinolytic proteins at the tPA and plasmin level. In addition, clot strengthening and modification can occur which render fibrin clot more resistant to plasmin degradation (e.g., TAFI cleaves lysine domains on fibrin). This measurement of clot weakening after 30 minutes is reflective of the patient's current systemic fibrinolytic function and increases specificity for identifying systemic pathologic hyperfibrinolysis. The strength of this assay includes all circulating factors that impact coagulation and fibrinolysis. Limitations of this assay are low-flow environment and lack of endothelial surface that can modify local fibrinolytic activity. There are currently no perfect assays for measuring fibrinolysis, but thrombelastography provides the most clinically relevant assay at this time to detect pathologic systemic hyperfibrinolysis

patients [18]. Raza et al. suggested that viscoelastic assays are limited to detect fibrinolysis impairment [37]. In their study, they identified a patient population without overt hyperfibrinolysis identified by ROTEM but with increased plasmin/antiplasmin (PAP) levels. These patients with high PAP, compared to patients with low PAP, and lacking hyperfibrinolysis by ROTEM had worse outcomes and higher mortality. PAP reflects localized plasmin production on fibrin, endothelium, and other surfaces with subsequent deactivation by complexing with circulating a2 antiplasmin. The half-life of this complex is 12 hours in healthy individuals [27]. Thus, elevation of PAP does not necessarily represent systemic fibrinolytic activity. D-Dimers, produced by plasmin degradation of fibrin, also have an extended half-life of 16 hours [27]. Thus, PAP and D-dimer levels are difficult to interpret as they may reflect systemic fibrinolysis shutdown or local suppression of fibrinolysis in an area of active fibrin breakdown. This same observation continues to be repeated in the literature in which investigators report high PAP [20] or D-dimer levels [21] with low levels of viscoelastic fibrinolytic activity and increased mortality. However, this same population with biomarkers of activation of the fibrinolytic system but low fibrinolytic activity does not have a gain in fibrin clot strength with TXA [38]. This patient population likely represents true fibrinolysis shutdown, in which at some point after injury there was systemic activation of the fibrinolytic system, but was subsequently shut down. This is consistent with observations in trauma patients dating back to the 1960s [39] in which fibrinolytic activity was

often suppressed early after injury despite elevated fibrin degradation products.

Viscoelastic assays are performed in a no-flow environment in which high levels of thrombin are rapidly generated, particularly when a potent activator is used, such as tissue factor. Maximum platelet activation can occur and, therefore, suppress fibrinolytic activity. Conversely, maximal platelet activation can result in platelet contraction followed by relaxation producing a pattern overestimating fibrinolytic activity. Therefore, the low values of LY30 (or high values of Ll60 for ROTEM) are compromised as estimates of systemic fibrinolysis.

Driver of Pathologic Fibrinolysis

Fibrinolysis May Not Be Linked to Blood Clot Formation in TIC

Coagulation changes after trauma are described to have two components: (1) impairment of blood clot formation (hypocoagulation) and (2) increased rate of clot degradation (hyperfibrinolysis) [40]. While one in four patients with severe injury has coagulation abnormalities within 30 minutes of injury, the prevalence of hyperfibrinolysis is much lower [33–35]. As the assessment of coagulation changes postinjury has become refined, hypocoagulation and hyperfibrinolysis appear mechanistically diverse. Principal component analysis suggests that hyperfibrinolysis does not correlate with impaired clot formation [41, 42]. This has also been evident using hierarchical clustering [43].

An interesting observation from human studies observed that blood taken from patients who have nontraumatic cardiac arrest have a high prevalence of hyperfibrinolysis [44]. There is compelling evidence that hypoperfusion drives hyperfibrinolysis, although others have argued that tissue factor from tissue injury may contribute to overactivation of systemic fibrinolysis based on experimental work [45]. Trauma patients have variable degrees of tissue injury and shock, and these collectively determine the different phenotypes of coagulation abnormalities. There may be unique patterns of injury that correlate to accelerated or inhibited fibrinolysis. Our recent animal work demonstrated that tPA levels were markedly increased in animals undergoing hemorrhagic shock, but tissue injury did not contribute to increases in systemic levels (Fig. 11.5). Interestingly, traumatic brain injury (nonherniation) appears to promote fibrinolysis shutdown [46, 47].

Currently, the only established risk factor for hyperfibrinolysis is inadequate tissue perfusion, which is supported by a number of retrospective

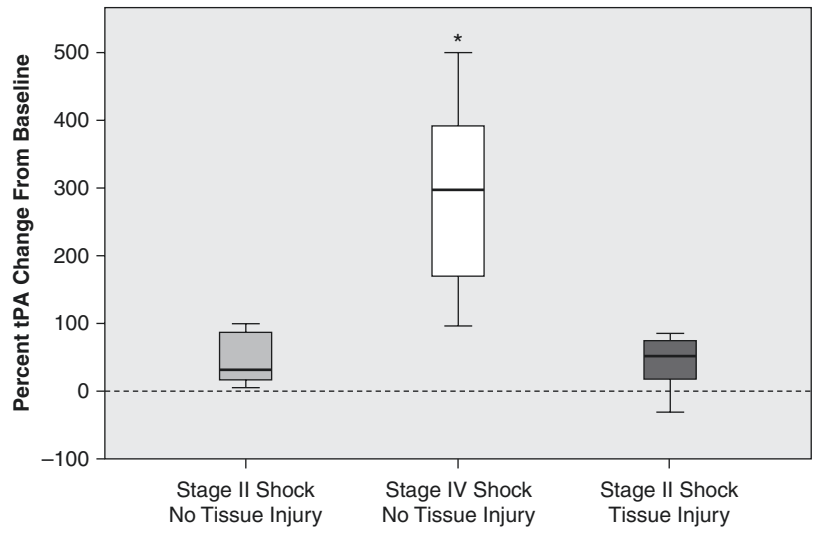


Fig. 11.5 In a rodent model evaluating tissue injury versus deep shock driving fibrinolysis, it was identified that tissue plasminogen activator is markedly elevated in response to shock, whereas tissue injury mildly increased changes and was comparable to control animals (stage II shock, no tissue injury)

studies [34, 40, 48, 49] and further strengthened by the fact that patients in the CRASH II trial who benefited from antifibrinolytics had a systolic blood pressure of less than 75 mmHg [50]. This is not a new concept, as investigators in the 1950s attributed fibrinolysis activation to ischemia and anoxia [51]. Shock may also be causing metabolic disturbances increasing fibrinolysis. It has been shown that taurocholic acid increases fibrinolysis in vitro and is markedly elevated following shock in rodents [52]. In sum, the molecular mechanisms of fibrinolysis in trauma remain an active area of research.

Hyperfibrinolysis

Pathologic hyperfibrinolysis was appreciated by Starzl et al. in 1963 [16] using thrombelastography (TEG) during the anhepatic phase of liver transplant surgery. In the original series of human transplant patients, these authors recommended empiric antifibrinolytics to reduce bleeding. However six years later, when evaluating the coagulopathy of liver transplant, Starzl's group [53] retracted their statement advocating empiric antifibrinolytics when they appreciated increased mortality from venous thromboembolism (VTE). Recognizing the potential for spontaneous resolution of excessive fibrinolysis is an important concept. In transplant, removal of the liver results in hyperfibrinolysis, and replacement of the liver corrects fibrinolysis without using medication to block fibrinolysis. The likely driver of fibrinolysis in liver transplant surgery is the removal of the liver, which has the ability to rapidly clear tPA through receptor-mediated processes [54]. Like most receptor-mediated processes, hepatic clearance of tPA can be saturated [55]. In trauma patients who develop TEG-detectable hyperfibrinolysis, tPA levels on admission are elevated compared to those without TEG-detected fibrinolysis [56]. The question in trauma remains if tPA generation or tPA clearance drives this process. It is likely that both contribute. Historic literature suggested systemic hyperfibrinolysis occurs early after injury, but proceeds to shut down after resuscitation [36, 57], indicating that after an acute phase of profibrinolysis, the body compensates by inhibition of the system.

The elevation of tPA in the plasma in trauma does not correlate to overall fibrinolysis activity. The cognate direct inhibitor of tPA, PAI-1, has been found to decrease in patients with TIC [58] and hyperfibrinolysis [56]. This relative drop in PAI-1 is most likely attributable to an increase in tPA, causing a complex of tPA and PAI-1 and, thus, decreasing the relative amount of free PAI-1 [59]. The source of early postinjury elevations of PAI-1 remains unclear as this protein may be released from a-granules of platelets and can be synthesized by endothelium. Furthermore, PAI-1 is an acute-phase reaction protein, and plasma levels do not peak for hours after hemorrhagic shock in animal models [60].

There are a number of additional proteins present in plasma that either inhibit tPA directly or can inhibit the effector protein of tPA, plasmin. Some of these proteins are normally in relatively high abundance in the plasma including alpha-2 antiplasmin, alpha-2 macroglobulin, alpha-1 antitrypsin, and C-1 esterase inhibitor. As described in Chap. 5, these are backup inhibitors of the fibrinolytic system, which will regulate fibrinolysis when tPA is elevated in the circulation. In addition to these potent plasmin inhibitors, thrombin-activatable fibrinolysis inhibitor (TAFI), generated by thrombin/thrombomodulin or directly released from platelets, inhibits plasminogen binding to fibrin. Plasma can buffer the effects of tPA, as is evident with previous experiments in which exogenous tPA mixed in whole blood with healthy volunteers required substantial concentrations to reproduce hyperfibrinolysis [61]. However, depletion of these backup fibrinolytic inhibitors of plasmin increase sensitivity to tPA-mediated fibrinolysis. Recently, it has been found that hyperfibrinolytic trauma patients are depleted of these inhibitors [38]. A2AP may be depleted by as much as 40%. In healthy volunteers, depletion of A2AP markedly increases tPA-mediated fibrinolysis [62] supporting the essential role of this potent plasmin inhibitor. A2AP can effectively inhibit free plasmin in fractions of a second [63] and free floating plasmin in systemic circulation is rapidly inhibited. This is

demonstrated by adding plasmin to whole blood, in which minimal fibrinolytic activity is evident until milligram levels of plasmin are added to whole blood, whereas nanogram of tPA can elicit significant fibrinolysis [62].

There is clear evidence that elevated tPA level with elevated fibrinolysis measured by VHA is associated with high mortality related to bleeding [20, 21]. However, patients with elevated PAP or D-dimer with low fibrinolytic activity have increased mortality that is typically delayed and not associated with life-threatening bleeding [20, 21]. The Gall et al. [21] study demonstrates this quite well as the low lysis high D-dimer patient cohort has a 4% rate of massive transfusion compared to 30% in patients with high fibrinolytic activity with elevated D-dimers [21]. Furthermore, the low lysis high D-dimer group had a median time to death of 2 days. Mortality beyond 24 hours in trauma is rarely associated with uncontrolled hemorrhage [64]. This "occult fibrinolysis" group also had the highest rate of deep vein thrombosis, supportive of a tendency towards thrombotic complications despite a prolonged prothrombin time. This group is much more consistent with fibrinolysis shutdown than hyperfibrinolysis.

The European group also proposed that the mechanism for ongoing undetected fibrinolysis was mediated by S100A10 [21]. S100A10 increases fibrinolysis via co-localization of the tissue plasminogen activator (tPA) with plasminogen by forming a heterotetramer complex with annexin 2 on a cellular membrane [65]. This is accomplished via the kringle domains on tPA and plasminogen which bind the lysine carboxy terminal of S100A10 known as the $p36_2/p11_2$ complex [66]. Two S100A10 monomers (forming the heterotetramer) are required to bring tPA and plasminogen in close proximity to effectively generate plasmin [66]. It remains unproven that circulating S100A10 monomers have the same impact on fibrinolysis. S100A10 monomers are essentially a protein version of tranexamic acid when not complexed with annexin A2; i.e., the fibrin binding sites of tPA and plasminogen are occupied by the $p36_2/p11_2$ domain preventing effective binding to fibrin carboxy lysine residues. The in vitro tests in the Gall study [21] support this hypothesis as increasing S100A10 in healthy volunteer blood decreased maximum lysis (ML) measured by ROTEM. Interestingly, this group may have found a previously unappreciated driver of acute fibrinolysis shutdown, with circulating S100A10 monomers binding tPA, reducing systemic fibrinolytic activity.

Fibrinolysis Shutdown and Hypofibrinolysis

Investigations of coagulation in elective surgery patients in the 1970s identified an increased risk of deep vein thrombosis (DVT) with low fibrinolytic activity following surgery [67], although this was not reproduced in other studies [68, 69]. Variable definitions and assays to define fibrinolysis shutdown were likely responsible for these inconsistencies. The euglobulin lysis time (ELT) that defines fibrinolysis shutdown was commonly used in coagulation research, but was known to have limitations [29]. Griffith [70] and Knight et al. [71] both demonstrated that prolonged ELTs postoperatively predict postoperative thrombotic complications. To add further confusion, the term hypofibrinolysis was introduced in 1974 [72]. This new type of impaired fibrinolysis was diagnosed by a lack of ELT shortening, or persistently elevated plasminogen activator inhibitor activity in blood samples obtained after venous occlusion of the upper extremity [73–76]. Hypofibrinolysis represents an impaired activation of the fibrinolytic system, whereas fibrinolysis shutdown is activation of the fibrinolytic system with subsequent inhibition beyond a physiologic level (Fig. 11.6). An important limitation in the current literature is assuming that hypofibrinolysis and fibrinolysis shutdown represent the same pathophysiology. Many clinic studies citing low fibrinolytic activity only include one measurement of fibrinolysis over time, which cannot effectively differentiate the two pathologies.

Impairment of the fibrinolytic system has a detrimental effect on patient outcomes. The mortality rate of patients with low fibrinolytic activ-

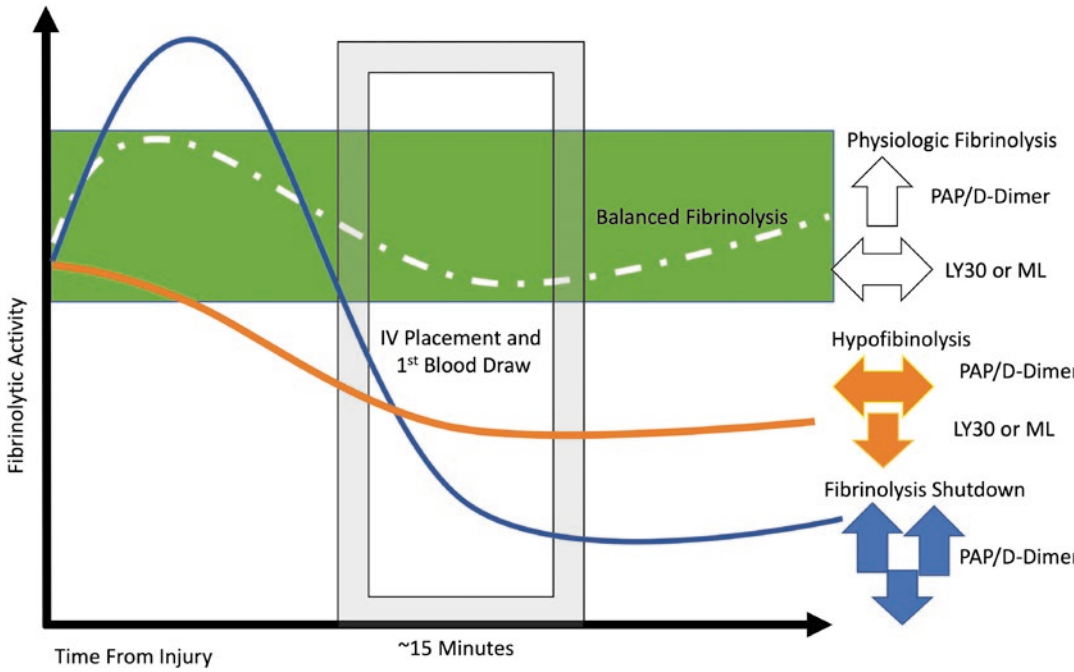


Fig. 11.6 Fibrinolysis shutdown vs hypofibrinolysis. The Y axis represents fibrinolytic activity, with a green bar representing a balanced level of fibrinolysis in which a patient has a fibrinolysis generation related to tissue injury and shock, a physiologic response to thrombin generation and tissue ischemia. The X axis represents time from injury. The gray box represents the first time a trauma patient is encountered by a prehospital provider and IV access is obtained, which in our urban setting is around 10–20 minutes following injury. At this time, laboratory analysis of a patient in fibrinolysis shutdown (blue) would demonstrate elevated D-dimer and PAP levels due to prior fibrinolysis activation, but have subsequently shut down the system, and viscoelastic assessment of fibrinolysis activity would be low. Those patients with physiologic fibrinolysis (white) due to their injury would have an elevated D-dimer and PAP level and a moderate amount of fibrinolysis measured by viscoelastic assessment. Patients lacking elevated D-dimer and PAP are either not severely injured, or have hypofibrinolysis, which is defined as a lacking activation of the fibrinolytic response to injury, which is a separate etiology than fibrinolysis shutdown and depicted in orange

ity on presentation to the hospital is nearly four times greater than patients with a physiologic level of fibrinolysis [77]. Death is attributable to organ failure. The term fibrinolysis shutdown was first used in 1969 [36] in a review on fibrinolysis describing the effects of electroplexy, myocardial infarction, and elective surgery. This transition was first described by Innes and Sevitt in 1964 [39]. As previously discussed, fibrinolysis shutdown and DIC were confused in their early description. Emerging evidence now suggests that DIC is a final common pathway after failure to treat the initial insult [78]. With the historic and current data, persistent fibrinolysis shutdown is emerging as a suspect for transitioning patients from TIC to DIC.

It is well known that trauma patients are prone to thrombotic events. When screening for postinjury venous thrombosis, the prevalence has been reported to approach 60% [79]. There is evidence for thrombosis in the pulmonary vasculature in nearly 1 in 4 seriously injured patients within 48 hours of their trauma [80]. Furthermore, microvascular clot is implicated in organ dysfunction [81, 82]. Therefore, it is intuitive that maintaining adequate fibrinolysis to clear the microvasculature of excessive fibrin deposition would be beneficial. The importance of inhibition of fibrinolysis at the site of injury remains essential during the acute phase of trauma, but systemic fibrinolysis shutdown is detrimental.

Inhibition of fibrinolysis measured by thrombelastography has been attributed to PAI-1 in severe sepsis [83]. LPS administration to animals increases PAI-1 levels over several hours [84] and similar observations have been made in humans that several hours after LPS injection fibrinolysis becomes impaired [85]. Acute lung injury models using mustard gas have attributed PAI-1 in addition to other antifibrinolytics (TAFI and A2AP) as culprits in progression to pulmonary failure [86]. Fibrinolysis inhibition is more common after trauma than hyperfibrinolysis. In 1991, Enderson et al. described that the majority of multisystem trauma patients in their study had elevated D-dimers and low fibrinolytic activity [87]. Raza et al. in 2013 showed minimal fibrinolysis activity measured by ROTEM and high plasmin-antiplasmin complexes in 57% of their patients [37]. It has been observed that 65% of severely injured patients have suppressed fibrinolytic activity measured by TEG within 12 hours of injury [77]. Subsequently low fibrinolytic activity measured by VHA has been associated with increased mortality in a number of major trauma centers in the United States [19, 20], in pediatric trauma patients [88], trauma patients in the intensive care unit [89], and internationally [21, 25]. The limitations of all of these studies is assuming low fibrinolysis shutdown. Future research is needed to differentiate these two dominant drivers of low fibrinolytic activity following injury.

Another important consideration in fibrinolysis shutdown is that these patients can still bleed for other reasons. Patients with low fibrinolytic activity and high D-dimer often harbor a prolonged prothrombin time and platelet dysfunction [21, 25, 90]. Therefore, ongoing bleeding cannot be completely attributed to fibrinolysis, and impaired clot formation appears to be the more likely culprit. The LY30 measurement in TEG relies on achieving maximum clot strength (~20 minutes) with a subsequent 30-minute delay. The role of a low LY30 to direct blood product resuscitation would be like putting the carriage in front of the horse. TEG indices to guide plasma, fibrinogen, and platelet are all obtained well before LY30 and before a D-dimer measurement. The survival benefit of viscoelastic goal-directed resuscitation over conventional coagulation assays has been validated in a prospective randomized trial [10], and LY30 or ML/CLI in ROTEM should be limited for clinical use in the decision of guiding tranexamic acid for bleeding if elevated, or in low activity levels to risk stratify patients for potential thrombotic events or organ failure.

There is also a time component to fibrinolysis shutdown, which is defined by acute, acquired, and persistent, which is extensively discussed in a recent review [91]. This will also be discussed in some detail on the fibrinolysis shutdown chapter (Chap. 32).

Treatment Strategies for Hyperfibrinolysis

In managing trauma patients at risk for pathologic fibrinolysis, it is important to first assess if there is active bleeding. Hemorrhage control should remain the priority for all trauma patients, as progression to shock will increase the likelihood of hyperfibrinolysis and ultimately death from uncontrolled bleeding. While hemorrhage control is in progress, resuscitation should be designed to be fibrinolysis neutral until the patient's fibrinolytic phenotype has been established. Currently, viscoelastic assays are clinically useful markers for detecting fibrinolytic activity to phenotype critically injured patients.

High volumes of prehospital crystalloid have been associated with hyperfibrinolysis [34] and supported by in vitro studies in which fibrinolysis was exacerbated by hemodilution of whole blood with saline and colloids [61]. Starch-based colloids may be problematic in resuscitating a patient at risk for hyperfibrinolysis as they have been implicated in impaired fibrin polymerization [92]. The role of permissive hypotension has a role in the prehospital setting as crystalloid dilution of antifibrinolytics (platelets and plasma) may lead to a hyperfibrinolytic phenotype. This may partially explain the benefit of delayed fluid administration reported by Bickell et al. in patients with penetrating torso trauma [93].

However, patients with inadequate perfusion are also at risk for increased tPA production and becoming hyperfibrinolytic. Patients in profound shock have been shown to benefit from prehospital saline [94]. The optimal prehospital fluid remains to be established (see Chap. 30 for more details on this topic). In our recent in vitro and animal studies, plasma resuscitation was found to attenuate hyperfibrinolysis. Successful resuscitation of a trauma patient will correct hyperfibrinolysis without antifibrinolytics. A case example of the beneficial effect of plasma first resuscitation in a hyperfibrinolytic patient is provided in Fig. 11.7. This was also appreciated in a prehospital plasma first resuscitation trial in which it was appreciated that one-third of hyperfibrinolytic trauma patients had corrected elevated fibrinolytic activity from their prehospital blood draw to emergency department arrival (<15 minutes) regardless of resuscitation strategy [95].

The use of empiric antifibrinolytics in trauma patients warrants careful evaluation. While the randomized CRASH II trial suggested a modest benefit in survival, the study's inclusion criteria were questionable [50] and the infusion of tranexamic acid (TXA) 3 hours after injury was associated with increased mortality [96]. The nonrandomized MATTERs study also suggested a survival benefit of TXA in the military setting, but with an increased rate of VTE [97]. There is also the confounder of the high rate of cryoprecipitate transfusions in patients who received TXA in this retrospective study, and many of these patients were resuscitated with colloids that have the potential to increase the risk of hyperfibrinolysis. None of these studies quantified fibrinolysis in patients being treated with TXA. The most recent military experience acknowledged that TXA was overutilized, was not associated with improved survival, and was associated with an increased rate of VTE [98]. A retrospective study from the United States reported that TXA use was associated with nearly twofold increased mortality in propensity-matched trauma patients [99]. Specific recommendations for antifibrinolytics will be addressed in Chap. 27 including

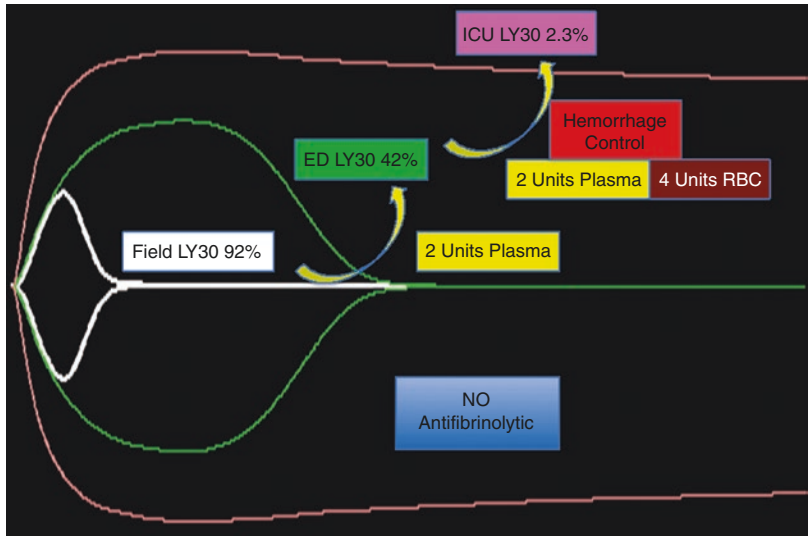


Fig. 11.7 Plasma-first resuscitation reversed a hyperfibrinolytic patient found by the paramedics with significant injuries, unresponsive, and profound shock (SBP 70 mmHg). This patient received two units of plasma en route to the hospital and increased his systolic blood pressure to 100 mmHg. His LY30 was reduced from 92% in the field to 42% upon arrival to the emergency department. The patient went emergently to the operating room where hemorrhage control was obtained. After receiving an additional two units of plasma and four units of RBC, the patient was transferred safely to the intensive care unit where his LY30 had returned to a physiologic range of 2.3%. He would be discharged from the hospital after a week and a few days with a full recovery. The patient required no antifibrinolytic medication and based on his field (prehospital) TEG had a > 90% predicted mortality

discussion on CRASH II, MATTERs I and II, and ongoing prehospital randomized trials using antifibrinolytics in trauma. However, there is no disagreement that antifibrinolytic agents should be targeted to the hyperfibrinolytic patient.

An important consideration when identifying patients who will benefit from antifibrinolytics to correct coagulopathy is to determine if they have elevated fibrinolytic activators with concurrent depletion of fibrinolytic inhibitors. There are at least two types of trauma patients with hyperfibrinolysis identified by VHA, one with depletion of fibrinolytic inhibitors (DFI) who have a mortality rate that exceeds 50% and those without depletion of fibrinolytic inhibitors with a mortality rate of 0% [38]. These non-DFI trauma patients had lower injury severity scores and minimal transfusion requirements. They likely have a physiologic response to injury in promoting fibrinolysis to keep clot formation localized to the site of injury. This may explain the previous observation that TXA based solely on LY30 does not reduce mortality [100]. Moreover, it remains unclear if TXA use off-target has adverse effects. Some evidence indicates that patients at highest risk of mortality are those with physiologic fibrinolysis who receive TXA, which is associated with an unadjusted increased mortality [101]. But with selective use of TXA in patients with DFI and hyperfibrinolysis, the potential survival benefit is greater than a 10% reduction in mortality [38]. There is a need for a randomized controlled trial to test empiric versus goal-directed TXA. While targeted TXA is limited to retrospective analysis, the trauma community should ask if it is content with a 1.5% reduction in mortality with empiric TXA use demonstrated in the CRASH II study, or if it desires goal-directed use with a survival advantage to improve this number almost tenfold.

Low Fibrinolysis Activity Does Not Equate to Hemostasis

It is important to take into consideration that fibrinolysis represents only one component of coagulation. Patients with low fibrinolysis can still bleed from their injuries due to coagulation changes impairing clot formation. It has even been proposed that fibrinolysis shutdown is a mechanism to attenuate bleeding when impaired clot formation is occurring [25] although this study was underpowered to prove this theory. Regardless, there is clear evidence that patients with elevated D-dimer or PAP levels with low fibrinolysis commonly harbor an elevated INR, lower fibrinogen levels, and decreased platelet count [20, 21, 38]. It remains unclear if these coagulation changes are concurrent with other drivers of TIC, or if prior plasmin activation has cleaved coagulation components, and this results in impairment of clot formation rather than fibrinolysis. As previously discussed TXA does not improve clot strength in this patient population [38], but goal-directed resuscitation [102] with platelets, cryoprecipitate, and plasma for specific blood components should be addressed to promote hemostasis in the bleeding trauma patient.

Blood products may also contribute to fibrinolysis inhibition. As previously discussed, platelets contain a number of potent antifibrinolytic proteins. While transfusion of platelets can be lifesaving, they have been associated with an increased risk for organ failure [103], which may be attributed to fibrinolysis shutdown. Cryoprecipitate may also contribute to fibrinolysis impairment as suggested in the MATTERs II trial [104]. Cryoprecipitate not only contains fibrinogen [105] but factor XIII [106] and fibronectin [107], which promote clots resistant to fibrinolysis. TXA use has also been associated with promoting and prolongation of fibrinolysis shutdown [108] and, if shutdown persists for more than 7 days, this has been associated with an eightfold increase in mortality [89].

The balance of blood product administration and risk for progression to fibrinolysis shutdown requires clinical judgement. If a trauma patient is not actively bleeding, the role of correcting laboratory-detected coagulopathy should be considered carefully. In the clinical scenario when large-volume hemostatic resuscitation occurs, an anticipated fibrinolytic shutdown should be anticipated upon arrival to the intensive care unit. Ongoing research is underway to determine if

these patients benefit from higher-intensity screening for thrombotic complications and if therapeutic interventions can be used to reduce thrombotic complications and organ failure in patients with fibrinolysis shutdown. For additional discussion on the treatment strategy of patients in persistent fibrinolysis shutdown to prevent organ failure and thrombotic complications, see Chap. 32.

Clinical Assessment of Fibrinolysis

The optimal test to determine systemic fibrinolysis status remains to be established. Euglobulin lysis has been considered to be the gold standard, but the important role of platelets in regulating fibrinolysis indicates that a whole blood assay is required. While D-dimers and PAP levels are sensitive in identifying the existence of fibrinolysis, they do not accurately reflect the current status of systemic fibrinolytic capacity. At this moment, TEG and ROTEM appear to be the optimal tests to identify fibrinolysis status in the injured patient. Retrospective studies from Denver [33] and Houston [34] have suggested that >3% LY30 are thresholds for treating hyperfibrinolysis with antifibrinolytic medication, as this appears to be the threshold for increased risk of mortality. However, these studies used different activators (kaolin versus tissue factor). A recent study has compared TEG and ROTEM values to define hyperfibrinolysis and fibrinolysis shutdown, which have good agreement using the rapid TEG LY30 and EXTEM ROTEM CLI60 and demonstrate the U shape distribution of mortality [22]. However, as previously mentioned there are subphenotypes within each of the 3 major phenotypes of fibrinolysis. A tissue plasminogen activator (tPA) TEG has been used to stratify these patients and has demonstrated that mortality and cause of death differ by fibrinolytic subphenotype [38, 109]. With the tPA TEG currently unavailable clinically, it is difficult to otherwise stratify these patient population. Thus, adjunctive laboratory measurement in addition to VHA is likely needed in the future.

It is important to appreciate that fibrinolytic activity measured by VHA can represent fibrinolysis shutdown or hypofibrinolysis. While the majority of trauma patients who undergo a massive transfusion with low fibrinolytic activity are in shutdown with evidence of elevated PAP levels [20], other injured patients may manifest with hypofibrinolysis. The use of D-dimer with VHA is most likely the most specific way to differentiate the groups, but there remains no standard for what an expected D-dimer or PAP level would equate to after injury and would require adjustment based on the amount of fibrin burden (~tissue injury). Animal models support that tissue injury do not increase fibrinolytic activity [110, 111] and that the combination of shock plus tissue injury modifies the fibrinolytic response to reduce fibrin degradation compared to hemorrhagic shock on its own [112]. The challenge in translating this to human subjects is controlling for the amount of fibrin generated from injury. Tissue injury producing fibrin will result in some degree of fibrin degradation and plasmin formation regardless of systemic fibrinolytic activity due to the biochemical properties of fibrin being a cofactor for plasmin generation [113]. More research is needed to define what differentiates hypofibrinolysis from fibrinolysis shutdown, as the current measurement of an LY30 of <0.9% persists as a predictor for increased mortality but may incorporate several populations of patients that would require divergent treatment strategies, which will be discussed in the fibrinolysis shutdown treatment (Chap. 32).

Conclusion

The fibrinolytic response to trauma can vary from low to hyperactivity. Low fibrinolytic activity is the most common fibrinolytic phenotype following severe injury, which has a reported prevalence of greater than 50%. It remains unclear whether low fibrinolysis following injury is predominantly due to fibrinolysis shutdown after prior activation of the fibrinolytic system, or whether the patient has a suppressed fibrinolytic response to injury. Hypofibrinolysis does not equal fibrinolysis shut-

down. Future research in trauma needs to differentiate these two pathophysiologies following severe injury. Hyperfibrinolysis on the other hand is relatively rare but associated with high mortality. Resuscitation fluids and blood products can alter the fibrinolytic phenotype. The goal of resuscitation should be fibrinolytic neutral until the phenotype can be determined. Antifibrinolytics should be selectively used in patients with confirmed hyperfibrinolysis and profound shock. The treatment strategies of low fibrinolytic activity to reduce thrombotic complications and organ failure in the intensive care unit is on the horizon. There are many questions to be answered in regards to the mechanisms that drive these processes.

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Complement System

12

Narcis I. Popescu and Florea Lupu

Introduction

One of the biggest challenges faced by multicellular organisms is the invasion of foreign pathogens; hence complex mechanisms that recognize, contain, and clear infectious agents have been evolutionarily developed. The response to invading pathogens is highly orchestrated and involves multiple immune, coagulation, and inflammatory pathways. Depending on the localization and the strength of the detected infectious signal, one or more of these pathways are sequentially initiated. In a simplified view, soluble or cellular receptors of the immune system are responsible for the recognition of the foreign agents, then the coagulation system isolates the threat from systemic distribution, and molecular and cellular immune effectors destroy and clear the pathogens. In addition, inflammatory mediators are responsible for fine quantitative tuning of the immune and coagulation responses to the level of pathogen threat and ultimately initiate the healing events after the clearance of infectious agents. Central to this physiologic response is a network of blood serine proteases grouped in several proteolytic cascades – the complement, coagulation, and fibrinolytic systems – which share a similar overall architecture and have developed from a common ancestral pathway. Integration between these cascades is achieved through multiple crosstalk mechanisms during an optimal response to foreign agents. Dysregulation of these networks, where the explosive potential of the proteolytic cascades is redirected toward self-structures, contributes to the pathophysiology of human diseases that have a strong inflammatory component. In this section we will review the architecture of the complement system, the interactions with the coagulation pathway, and its role in hemostasis and development of prothrombotic human diseases.

Complement Organization and Function

The complement system is one of the earliest defense mechanisms developed during the evolution of animal kingdom [1, 2]. Its primary role is the rapid identification and clearance of microbial intruders [3, 4]. As shown in Fig. 12.1, complement system includes a large number of components, both soluble and cellular effectors, and an array of regulatory proteins that modulate the activation and propagation of complement

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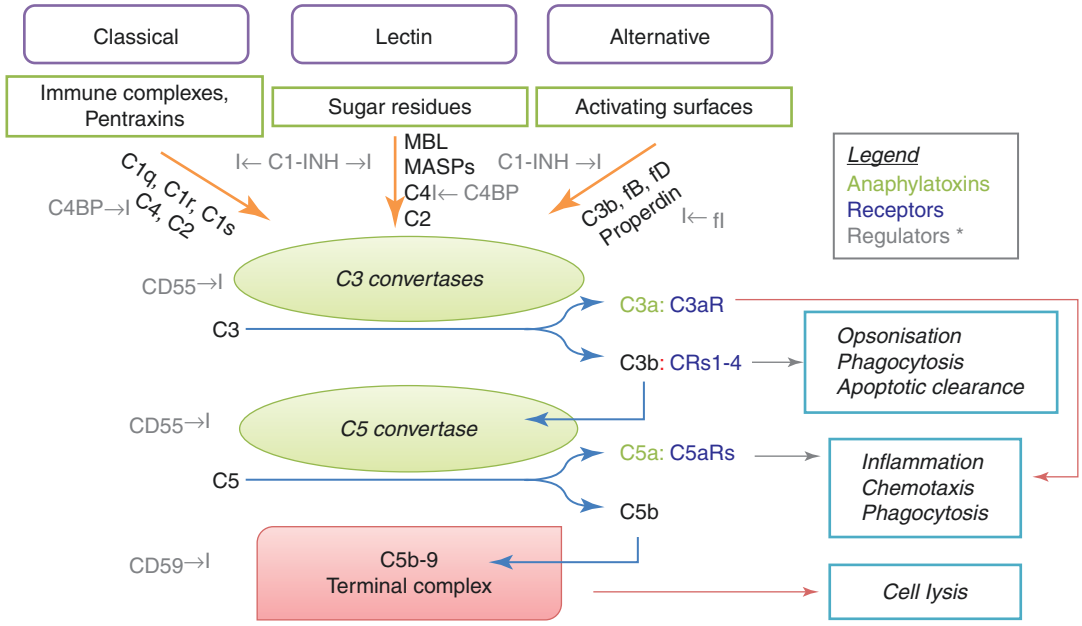


Fig. 12.1 Molecular pathways and mechanisms controlling complement activation and its physiological and pathological effects

reactions [5]. Three distinct pathways of complement activation have been characterized to date: classical, lectin, and alternative pathways (CP, LP, and AP, respectively). All three pathways share a similar overall architecture. Preformed complement components, present as zymogens, are activated in response to initiating events to generate effector serine proteases that cleave and activate other downstream proteases. This evolutionary design of cascaded biochemical reactions allows a fast response to intruding pathogens without the need of *de novo* synthesis. Moreover, this permits an explosive amplification of the signal since any upstream enzyme can proteolytically cleave multiple downstream zymogen substrates, which themselves become effector proteases. The proteolytic cascade underlying blood coagulation shares a similar design and is detailed in another chapter of this book. The three activation pathways converge at distinct points in the complement cascade, ultimately leading to the deposition of C5b on pathogenic surfaces. C5b deposition initiates the terminal complement pathway, the sequential formation of a multimeric membrane-spanning complex with cytolytic antimicrobial function. In contrast to the activating pathways, the terminal pathway does not require proteolysis of complement components. Furthermore, small peptides released during the proteolytic activation of complement components interact with cell-associated receptors and initiate downstream signaling events that modulate the immune and inflammatory responses. The activation and propagation of the complement response is tightly controlled by multiple soluble and cell-associated regulators, which ensure that the complement is primed to act on intruding pathogens but does not attack self-structures.

Analysis of complement deficiencies in human diseases [6] revealed that the primary role of the complement system is the rapid recognition and removal of intruding pathogens. In addition, targeted manipulation of the complement system in experimental models, genetic associative studies, and, more recently, the use of selective inhibitors against complement effectors have revealed new and sometimes unexpected roles for the complement system. As such, the complement components play a direct role in the

phagocytosis of opsonized pathogens by macrophages and dendritic cells [7], initiate and direct adaptive immune responses [4], contribute to the noninflammatory clearance of apoptotic cells and the removal of antibodies against self-antigens [8], and shape the natural antibody repertoire [9]. In tantalizing recent developments, the complement's potential to clear cellular processes has been harnessed for synaptic remodeling and maintenance of homeostasis within the central nervous system [10, 11]. Conversely, excessive complement activation can have deleterious systemic effects associated with increasing numbers of human pathologies with strong inflammatory components, such as autoimmune diseases [12], sepsis [13], and thrombotic microangiopathies [14], among others. Early complement activation following trauma [15, 16] is primarily initiated by damaged self-structures [17], can be amplified by penetrating pathogens due to impaired barrier function, and associates with poor prognosis for these patients [18, 19].

Complement Activation Pathways

The complement system (Fig. 12.1) can be activated through three distinct pathways: classical, lectin, and alternative pathways [20]. All three pathways contribute to an optimal complement response, although they recognize different pathogen-associated molecular patterns (PAMPs), are initiated by distinct pattern recognition molecules (PRMs) or structures, and use different molecular components. Furthermore, the same PAMPs can activate multiple PRMs, leading to redundant activation of complement pathways. The three activation pathways intersect at distinct points throughout the proteolytic cascade, either the C3 or C5 convertase complexes, and elicit similar downstream biologic effects.

The classical pathway of complement activation, despite being the first characterized, is probably the latest developed evolutionarily [1] and takes advantage of the antibody recognition of foreign antigens. Multimeric IgM immunoglobulins, or clusters of IgG immunoglobulins, interact with the C1 complement component and initiate complement activation. C1 is a pentameric complex containing one C1q subunit and two subunits each of C1r and C1s. C1 interaction with immunoglobulin-antigen complexes induces a conformational change in the C1q PRM, followed by C1r autoactivation and C1s transactivation [21]. C1s is the effector protease of the classical pathway initiating complex and cleaves the complement protein C4 into the C4a and C4b fragments. C4b has enhanced reactivity and binds to surfaces in the vicinity of the complement activation site leading to their opsonization. The complement protein C2 binds C4b and is cleaved by C1s, generating the classical pathway C3 convertase complex C4b2a. C4b2a localizes the proteolytic function of the C2a subunit to surfaces opsonized by C4b. This C3 convertase subsequently cleaves complement protein C3, which is responsible for amplification and downstream effects of the complement activation.

The classical pathway can also be initiated by antibody-independent mechanisms induced by acute phase PRMs such as the pentraxins PTX3, C-reactive protein (CRP), and serum amyloid P (SAP) bound to specific PAMPs [22]. Upon interaction with C1, they similarly induce conformational changes in C1q that support activation of C1r and C1s, subsequent proteolytic cleavage of C4 and C2, and generation of the C3 convertase C4b2a.

The lectin pathway of complement activation is usually initiated by carbohydrate structures on the pathogen surface or acetylated patterns from damaged self. These PAMPs are recognized by PRMs from the collectin family, such as mannose-binding lectin (MBL) [23], ficolins (Ficolin-1, 2, and/or 3) [24], and other collectins (CL-L1, CL-K1, CL-P1) [25], usually found in complex with three serine proteases, termed MBL-associated serine proteases (MASP-1, 2, and 3). Similar to the classical pathway, PAMP recognition by collectin PRMs leads to autoactivation of MASP-1, and/or the alternatively spliced isoform MASP-3 [26], which in turn transactivates MASP-2 [27]. MASP-2 is the critical protease from the lectin pathway-initiating complex since it is the only one that can cleave C4 required for

the C3 convertase complex [28]. C2 bound to C4b at the site of activation is cleaved by both MASP-1 and MASP-2 [29]. This generates the C4b2a complex, identical to the C3 convertase initiated by the classical pathway.

The alternative pathway is considered the oldest evolutionarily and the least specific pathway of complement activation. A tick-over mechanism in which C3b is constantly generated in plasma in sufficient amounts to interact with factor B (FB) and generate a fluid-phase C3 convertase [30] supports the basal activation of the alternative pathway. Additionally, nucleating C3b can be formed by spontaneous hydrolysis of C3 [31, 32], via C3 convertases formed by the other two complement activating pathway, and to a smaller extent by vascular proteases such plasmin [33] or coagulation proteases FXa, thrombin (FIIa), FIXa, or FXIa [34]. FB subsequently binds C3b and is activated by the serine protease factor D (FD) resulting in the formation of the alternative pathway initiating complex C3bBb, which retains proteolytic activity toward C3. This initiating complex is stabilized on activating surfaces by properdin (P) and forms the C3 convertase C3bBbP. Through a positive feedback amplification more C3b is generated which opsonizes the target surface, can form new alternative pathways initiating complexes with FB, or can attach to the C3bBb and form the C3bBb3b convertase with proteolytic activity toward C5. It has been estimated that the alternative pathway amplification, and implicitly the C3bBb3b C5 convertase, accounts for the overwhelming majority of C5 activation even when the complement cascade is initiated by the classical or lectin pathways [35].

Regardless of the molecular pathway involved, activation of the complement system leads to the proteolytic cleavage of C3 and generation of two biologically active fragments, C3a and C3b. C3a, a small 77-residue polypeptide, is one of the complement anaphylatoxins that modulates downstream immune and inflammatory responses through engagement of C3a receptors (C3aR) present on multiple cells [36]. C3b is the larger fragment released by C3 cleavage and functions as an opsonin, tagging molecular structures for subsequent immune response and clearance. During C3 activation, a highly reactive thioester bond is exposed on the C3b fragment, which allows the covalent attachment to vicinal proteins and carbohydrates through ester or amide linkages [37]. In contrast to native C3, C3b also exposes binding sites for other complement components: FB and properdin, which contribute to the generation of the alternative pathway C3 convertase in the presence of FD; complement protein C5, critical for downstream terminal complement pathway; cellular complement receptors important for opsonin-induced phagocytosis; and negative regulators (the protease factor I, FI) and cofactor proteins (factor H, FH; membrane cofactor protein, MCP; decay-accelerating factor, DAF) that inactivate C3b. Despite showing enhanced affinity for binding carbohydrates, C4b and IgG [37], C3b cannot discriminate between self and nonself-structures and can opsonize host cells as well. The local balance between negative regulators (FI, DAF) and downstream effectors (C5, FB, P) controls the outcome of C3b deposition, resulting in either amplification or inactivation of the complement cascade. Host cells express cofactors on their surface, which enhance C3b proteolytic inactivation by FI, and thus are normally protected from complement attack. On pathogen surfaces however, C3b inactivation is slower in the absence of cofactors, which leads to local amplification of the complement cascade and further deposition of C3b on the multimeric C3 convertases. The resulting convertase complexes, either C4b2a3b or C3bBb3b, shift their substrate preference toward C5 conversion and initiate the terminal complement pathway.

Terminal Complement Pathway

The proteolytic cleavage of C5 is the first step of the common downstream effector pathway responsible for the generation of the terminal complement complex on microbial surfaces. Proteolytic activation of C5 on complement opsonized surfaces generates a small anaphylatoxin, C5a, and a larger fragment C5b, which initiates the formation of the terminal comple-

ment complexes (TCC) [20, 38]. Independent of complement convertases, vascular proteases like plasmin and FXa, and to a lower extent thrombin, could support C5 activation, releasing the C5a anaphylatoxin and fluid phase C5b [33, 34, 39, 40]. Albeit this alternative C5 activation is minor when C3 is present [39], it could support terminal complement pathway activation in pathologic conditions with severe complementopathies, such as sepsis and trauma [41]. C5b then sequentially binds to complement proteins C6 and C7, forming a stable C5b-7 trimer that exposes lipid-binding sites for insertion into biological membranes. C5b-7 recruits complement protein C8 and generates the C5b-8 tetramer, which in turn promotes binding and polymerization of multiple C9 subunits into a tubular structure forming the C5b-9 pore complex [42, 43] called the membrane attack complex (MAC). While C5b-8 can form transient lytic pores in mammalian cells [44, 45], stable C5b-9 pores (MAC) are required for complement mediated killing of bacterial pathogens [46].

Similar to C3b deposition, the TCC intermediaries, C5b-7 to C5b-9, do not have an intrinsic ability to discriminate between self and non-self surfaces and have the potential to bind to host cells as well. To protect against damaging complement attacks on self-structures, multiple mechanisms co-developed evolutionarily, such as the ubiquitous expression of cell surface CD59 [47, 48] which blocks propagation of complement reaction on host cells. Similarly, circulating vitronectin [49] or clusterin [50] interacts with soluble C5b-9 (sC5b-9) complexes and blocks its insertion into biological membranes. Nonetheless, non-lytic concentrations of sC5b-7/8/9 are able to activate signaling cascades in endothelial cells, lymphocytes, and smooth muscle cells [51] and thus contribute to an integrated complement-coagulation response at sites of injury, as detailed later in this section.

Complement Receptors

Complement activation exerts multiple downstream biological effects, not only through its terminal effector arm, the cytolytic C5b-9 complex, but also through complement receptors that engage intermediaries generated throughout the cascade. There are two main groups of complement receptors: opsonin receptors, which recognize C4b and C3b opsonins and their proteolytically inactivated products, and anaphylatoxin receptors.

The opsonin receptors, such as complement receptors CR1, CR2, CR3, CR4, the complement receptor of immunoglobulin family (CRIg), and C1q receptors [3, 5, 52], usually promote phagocytosis of complement-opsonized structures. On specialized cells these receptors induce complementary signaling events that contribute to the adaptive immunity and/or inflammatory response. CR1 (CD35) is widely expressed and versatile, recognizing all major complement opsonins, C1q, C3b, and C4b [53], as well as their inactivated forms iC3b and iC4b. CR1 is exposed on the surface of erythrocytes, neutrophils, monocyte/macrophages, and subsets of B and T lymphocytes and follicular dendritic cells (FDCs) [5]. On professional phagocytes, CR1 stimulates phagocytosis of opsonized structures, while on erythrocytes CR1 is critical for the clearance of circulating immune complexes [54]. CR1 is a negative regulator of complement convertases on host cells and also functions as a cofactor for FI-mediated proteolytic inactivation of C3b and C4b [55]. Furthermore, CR1 modulation of CR2-dependent signaling events in B lymphocytes and FDCs [56] is critical for optimal adaptive immune responses [57].

CR2 (CD21) is a complement receptor expressed mainly on immune cells such as B lymphocytes, FDCs, thymocytes, and some peripheral T cells [5]. CR2 binds C3b and the proteolytically inactivated forms iC3b, C3dg, and C3d on opsonized surfaces and regulates downstream adaptive immune responses. In addition, CR2-mediated events play a crucial role for the clonal selection of B1 lymphocytes and development of the natural antibody repertoire, regulation of B cell tolerance, and maintenance of long-term B cell memory [4, 58, 59].

CR3 (CD11b/CD18) and CR4 (CD11c/CD18) are complement receptors from the β2 integrin

family present mainly on neutrophils, NK cells, monocytes, activated T lymphocytes, and the specialized liver macrophages Kupffer cells [60]. CR3 and CR4 enhance phagocytosis of iC3b-opsonized particles [3, 5]. These β2 integrin receptors also have complement-independent functions in leukocyte interaction with vascular cells and integrin-mediated signaling. The complement receptor of the immunoglobulin family (CRIg) is expressed by Kupffer cells; interacts with C3b, iC3b, and C3c fragments; inhibits AP C3/C5 convertase activity; and plays a major role in the clearance of C3-opsonized particles [61, 62].

The anaphylatoxin receptors recognize small peptides released during complement activation, namely, C3a, C4a, and C5a [36]. Three anaphylatoxin receptors, belonging to the G protein-coupled receptor (GPCR) class, have been characterized so far: the C3a receptor (C3aR) and two C5a receptors, C5aR1 (C5aR, CD88) and C5aR2 (C5L2) [52]. These receptors are expressed by a wide variety of cells and usually induce proinflammatory outcomes [36, 63, 64]. While C3aR and C5aR1 induce productive signaling events in target cells, C5L2 is uncoupled from cytosolic G protein effectors [65]. C5aR2/C5L2 is currently considered a decoy receptor that modulates the C5a-C5aR1 signaling events [64]. C4a has been recently shown to bind protease-activated receptors (PAR) 1 and 4, promoting endothelial activation, cytoskeletal reorganization, and enhanced permeability of endothelial monolayers [66]. PAR1/4 receptors are expressed on other vascular cells as well and are critically important for thrombin-mediated platelet activation [67]. As such, it is expected that C4a peptide could also promote or modulate platelet responses in clinical settings with concomitant activation of complement and coagulation cascades, such as sepsis and trauma.

The GPCR anaphylatoxin receptors have pleiotropic proinflammatory effects. Anaphylatoxins are strong chemoattractants for monocytes/macrophages, neutrophils, activated B and T lymphocytes, basophils, and mast cells [64]. They are powerful vasodilators of small blood vessels and promote leukocyte adhesion to endothelium, as well as immune cell recruitment and cell migration toward sites of infection, where they trigger oxidative burst in macrophages, neutrophils, and eosinophils, degranulation of neutrophils, and release of soluble mediators from basophils, mast cells, and platelets [36, 63, 64]. In addition, anaphylatoxin receptors initiate multiple cell signaling pathways, including PI3k/Akt, MAP kinase, phospholipase C, and NF-kB pathways, inducing a proinflammatory transcriptome and production of multiple chemokines and cytokines such as TNF-α, IL-1β, and IL-6, among others [63]. Excessive C5aR signaling however can have deleterious effects, such as paralysis of neutrophil immune functions [39], thymocyte apoptosis [68], and/or induction of consumptive coagulopathy [69, 70].

Complement Regulation

Similar to other proteolytic cascade systems, such as the coagulation, the optimal complement response is maintained through a fine balance between activators and inhibitors of the complement system. This design ensures that the complement cascade is primed to react to invading pathogens while limiting its deleterious effects on host cells, which are usually protected through surface expression of negative complement regulators. Complement regulation acts at multiple steps throughout the complement cascade and includes inhibitors of complement activation and/or propagation convertases, proteases that inactivate complement effectors, cofactors and allosteric modulators, as well as clearance receptors [3–5]. Sometimes the same regulator can act through multiple mechanisms, such as the CR1 complement receptor discussed above, which is a competitive inhibitor of complement convertases, an allosteric cofactor for proteolytic inactivation of opsonins, and a clearance receptor of opsonized surfaces.

Initiation of the complement cascade is maintained silent by the C1 esterase inhibitor (C1-INH), an abundant vascular serpin. C1-INH is the primary regulator of effector serine proteases from the classical [71] and lectin initiating

pathways [72] and to a lesser degree the alternative pathway [73]. C1-INH has a broad spectrum toward vascular serine proteases, inhibiting also coagulation (kallikrein, FXIIa, FXIa) and fibrinolytic (tissue plasminogen activator, tPA, and plasmin) enzymes, thus concomitantly regulating multiple hematologic responses [74]. Lower C1-INH activity in plasma due to proteolytic inactivation of the serpin has been reported in sepsis [75] and therapeutic infusion of C1-INH protected against sepsis-induced complementopathy, but not coagulopathy, in experimental models [76] and improved all-cause survival in a recent human trial [77]. A recent analysis of multiple trauma patients, albeit underpowered, also identified lower C1-INH activity as a negative prognostic factor in these patients, even in the absence of overt DIC [78].

If complement activation progresses past the initiation phase, proteolytic inactivation of the C3b and C4b opsonins, mediated by complement factor I (FI), controls the amplification phase of the cascade [79]. FI cleaves C3b and C4b opsonins either in solution or on activated surfaces, and its enzymatic activity can be accelerated by soluble and cell surface cofactors. Complement factor H (FH) is a soluble decay accelerator of the AP C3 convertase (C3bBb) and a cofactor of FI-mediated proteolysis of C3b [80], while C4BP, C4b-binding protein, is a soluble decay accelerator of the CP/LP C3 convertases and a cofactor of FI-mediated proteolysis of C4b [81]. FH and C4BP protective functions are concentrated on host cells through specific interactions with surface carbohydrate structures such as sialic acid and glycosaminoglycans. In addition, complement C3b/C4b receptors (CR1, CR2), C2 receptor inhibitor trispanning (CRIT) [82]), DAF (CD55), and the cell surface cofactor MCP (CD46) can bind deposited C2, C3b, and/or C4b, restricting their availability for the formation of C3/C5 convertases and/or enhancing FI-mediated opsonin proteolysis on the surface of host cells [48]. Furthermore, the surface-exposed CD59 blocks propagation of TCC complexes on host cells, while sublytic C5b-9 deposits are eliminated by endocytosis or membrane vesiculation [83]. As mentioned earlier, vitronectin and clusterin bind fluid-phase C5b-9 and inhibit its insertion into host cells. Finally, circulating carboxypeptidases convert complement anaphylatoxins into their desarginated forms (C3a-desArg and C5a-desArg), which either impair or shift the signaling response mediated by C3aR/C5aR1 receptors [36].

Given these overlapping regulatory mechanisms, complement is initiated locally on pathogen/PAMP surfaces or on damaged self-structures due to the lack of, or reduced presence of, protective complement inhibitors. The controlled activation of complement recruits immune cells at injury sites, tags non-self and damaged self-structures, promotes their clearance, and primes repair mechanisms. If regulatory mechanisms fail to contain complement activation, systemic dissemination of complement mediators (anaphylatoxins, opsonins, TCC) occurs, resulting in systemic inflammatory responses, complement deposition, and activation of proteolytic cascades at distant sites, ultimately leading to multiple organ dysfunction and death.

Integrated Hematologic Response to Injury

Vascular trauma, defined as the disturbance of vascular homeostasis, could be initiated by multiple types of injuries: infections, biomechanical injuries, and/or exacerbated inflammation (Fig. 12.2). Despite their initiating diversity, they are all resolved through similar effector pathways involving immune (complement), hemostatic (coagulation/fibrinolysis), and inflammatory responses. Due to their concerted action, these pathways are closely intertwined and are co-regulated by common molecular mechanisms. Together they form a dynamic integrated response to injury aimed at stabilizing the damaged site, resolving the injury, and repairing the organism. While here we refer to vascular disturbances for ease of exemplification, extravascular injuries also require a hematologic response for the recruitment of molecular and cellular immune effectors, their transport across the vascular wall, and the coordinated protection against the loss of vessel integrity at extravasation sites.

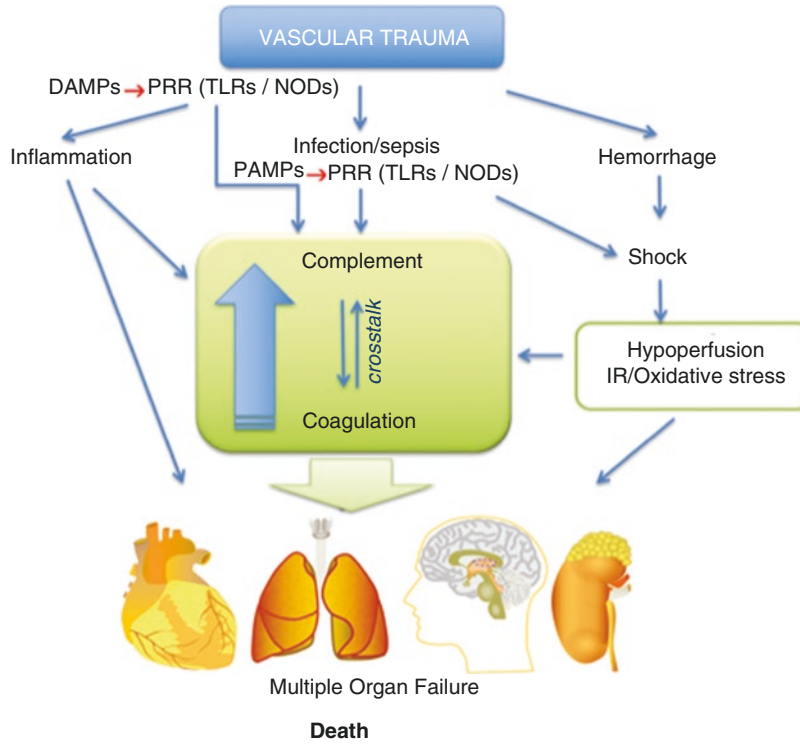


Fig. 12.2 Role of the complement-coagulation crosstalk in the pathophysiology of sepsis and trauma

The optimal hematologic response has several characteristics: (1) it is designed to *act locally* at sites of injury, (2) is *discrete* and *progressive*, and (3) is *reversible*. The first two characteristics are a direct consequence of the abundance of negative regulators/inhibitors of the initiation pathways; the reversibility of the hematologic response is ensured by balancing proteolytic cascades (fibrinolysis) and/or tissue remodeling pathways and is needed to restore the functionality of the organism. The hematologic response could be initiated by the complement system at sites of infection or by the coagulation system at sites of vessel wall damage. At these sites, the presence of danger signals, pathogen PAMPs, or host organism-derived damage-associated molecular patterns (DAMPs) enhances the proteolytic activity of initiating proteases and tips the balance toward initiation/amplification of the hematologic response. The proteolytic response is maintained for as long as the initiation/amplification factors exceed the counteracting effect of the inhibitors; hence the response is *discrete* and does not use all components present systemically. If enough danger signals are detected, the proteolytic cascade *progresses* to the amplification phase, which further recruits molecular and cellular effectors and engages complementary response systems, such as inflammation. In the meantime, clearance of initiating dangers diminishes the proteolytic amplification phase and tilts the balance toward inhibition of the hematologic response. After the infection and/or vessel damage is resolved, remodeling mechanisms, such as the fibrinolytic system, are activated and *reverse* the affected areas to their prior functionality. In contrast, when the hematologic response losses one or more of its defining characteristics, such as systemic and/or exacerbated activation due to failure of regulatory mechanisms, its explosive proteolytic potential can have damaging effects on the host, leading to multiple organ failure and death. In this section we will focus on the interplay between the proteolytic cascades that usually initiate the hematologic response to injury, namely, the crosstalk between the complement system and coagulation.

Architectural Similarities Between the Coagulation and Complement Systems

The coagulation system is detailed in another chapter of this book; therefore, we will only highlight its similarities with the complement here. The coagulation system is closely related to the immune proteolytic system (complement) evolutionarily, architecturally, and functionally, and they are intimately connected during the initiation of the hematologic response. Evolutionary analysis revealed that the coagulation cascade has derived from a proto-immune proteolytic system, similar to the complement system, through acquisition of unique structures and specialized functions [84]. Molecular analysis of the invertebrate immune system from the Limulus species revealed a proteolytic system with both opsonic (complement) and clotting (coagulation) functions [85, 86]. This is thought to represent the ancestral proteolytic cascade before the divergence of the two systems. Interestingly, the current FDA-approved test for endotoxin/pyrogen detection (Limulus amoebocyte lysate, LAL, test) takes advantage of this dual functionality. In the LAL test, lysates of Atlantic horseshoe crab (*Limulus polyphemus*) hematocytes (amoebocytes) containing the proto-immune cascade are incubated with the test sample, whereby the endotoxin initiates the lectin complement pathway and leads to opsonization as well as clot formation, which is the endpoint readout of the assay.

Analogous to the complement system, coagulation is organized as a cascade of serine proteases that are constitutively circulating in blood as zymogens. Coagulation can be initiated by the intrinsic, or contact, pathway or by the extrinsic, TF-dependent, pathway. Similar to complement activation, the contact pathway is initiated by the conformational autoactivation of factor XII on exogenous surfaces [87], such as negatively charged polyanions [88] and/or microbial surfaces [89]. In contrast, the extrinsic pathway is initiated upon exposure of a danger-associated molecule, tissue factor (TF), to circulating factor VII(a) [90]. TF is the main physiologic initiator of blood coagulation and is normally restricted from exposure to blood. It is constitutively expressed in mural and adventitial layers of the vessel wall; thus exposure of TF to circulating coagulation factors is usually associated with the loss of vessel wall (endothelial) integrity. Due to its critical role in blood clotting, TF is an important node for the functional convergence of complement and coagulation systems [13, 91, 92], and inflammatory mediators regulate its vascular expression, as reviewed below.

Similar to complement, the two blood clotting pathways converge at factor Xa (FXa) activation, which, in the presence of cofactor Va (FVa), generates thrombin (FIIa) through proteolytic cleavage of zymogen prothrombin. Thrombin is the final effector protease of the clotting cascade, which converts soluble fibrinogen into insoluble fibrin that provides the matrix for blood clots. Both FXa and thrombin are involved in positive feedback amplification of the clotting cascade and could support complement C3 and C5 activation, as well as inflammatory responses through engagement of protease activated receptors (PARs) in the vasculature.

Like the complement cascade, the proteolytic potential of the coagulation cascade is repressed by multiple regulatory mechanisms. Coagulation only progresses if the local activator signals overcome the negative regulators. C1-INH, the main inhibitor of the classical and lectin complement activation pathway, also controls the initiation of the contact clotting pathway. Thus C1-INH represents a direct molecular bridge between the complement and coagulation cascades, ensuring their concomitant regulation. The initiation of blood clotting through the extrinsic pathway is controlled by tissue factor pathway inhibitor (TFPI), a serpin present on endothelial cells and activated platelets, the main cellular entities associated with the propagation of the clotting cascade [93]. TFPI, similar to surface-associated complement regulatory proteins, ensures that coagulation is not initiated on undisturbed host cells. Antithrombin III (ATIII) is the main soluble inhibitor of serine proteases in the amplification/termination phase of blood coagulation (FIIa > FXa). Like C1-INH, ATIII exhibits broader

substrate specificity, inhibiting less efficiently upstream coagulation factors (FIXa, FXIa, FXIIa) [94, 95] as well as MASP-1/2 proteases of the lectin pathway of complement activation [96]. Other regulators of blood coagulation, such as the protein C (PC)-thrombomodulin (TM) axis, are discussed in detail in other chapters of this book.

Crosstalk Between the Complement and Coagulation Cascades

The molecular interactions between the complement and coagulation cascades are multiple and complex, and the crosstalk can either be direct, whereby components of one cascade interact with members of the complementary system, or indirect, through engagement of inflammatory responses and cellular mediators. Inflammatory responses seem to be the preferred communication conduit for the integration of hematologic responses to vascular trauma, controlling the level of activation as well as the progression of the response.

Complement activation induces a prothrombotic environment by augmenting the procoagulant function of clotting factors or decreasing the anticoagulant regulatory mechanisms. Some of the underlying molecular mechanisms are highlighted in Fig. 12.3. In addition, signaling initiated by complement anaphylatoxins or soluble TCC intermediaries fosters recruitment and/or activation of common cellular effectors, such as monocytes, platelets, and endothelial cells. For example, upstream complement protease MASP-1, activated on microbial surfaces by an MBL-dependent mechanism, promotes a local prothrombotic environment through direct activation of prothrombin [97]. The resulting throm-

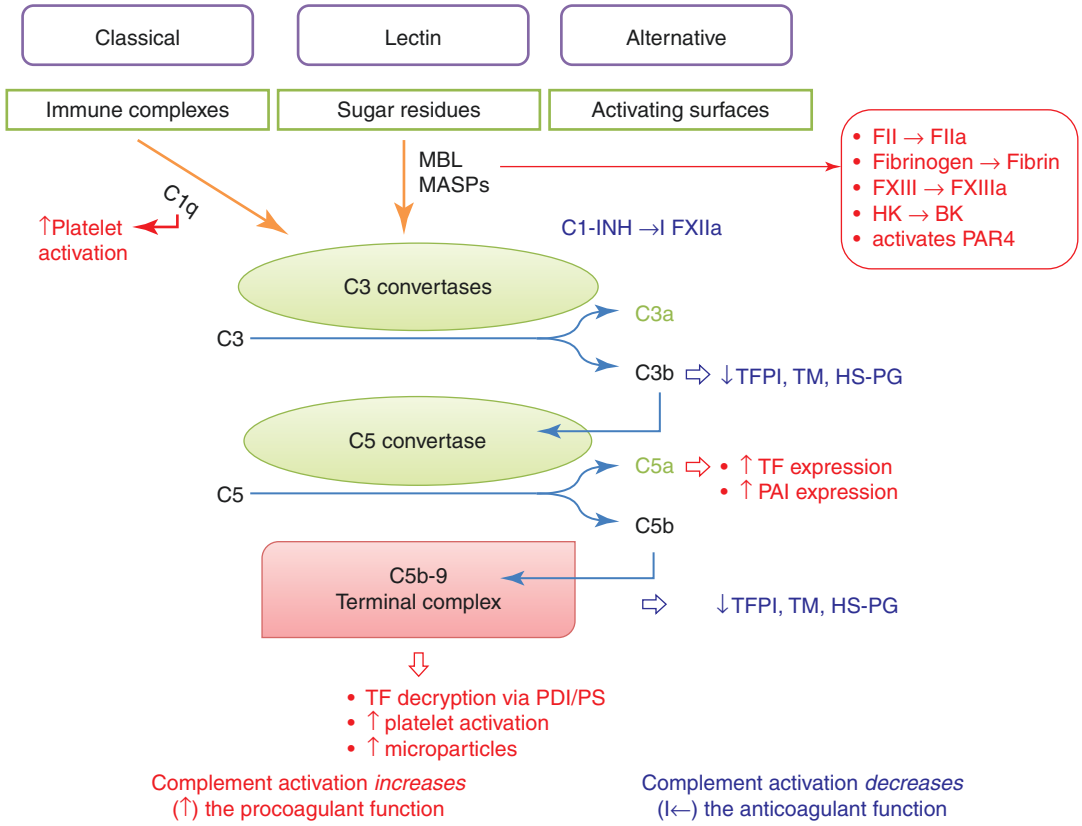


Fig. 12.3 Interactions between complement proteins and the hemostatic system

bin primes the coagulation cascade, through activation of FV and FVIII cofactors, and amplifies prothrombotic and inflammatory responses through PAR signaling. In addition, MASP-1 could activate thrombin substrates directly, such as plasma transglutaminase factor XIII (FXIII), fibrinogen, and thrombin-activatable fibrinolysis inhibitor (TAFI), and as a result helps stabilize the clot and delay its lysis at infection sites [29, 97, 98]. Activated FXIII (FXIIIa) crosslinks fibrin fibrils within the clot [99], while TAFI inhibits fibrin-dependent fibrinolytic events initiated by tissue plasminogen activator (t-PA) [26]. While MASP-1 does not cleave fibrinogen similarly to thrombin [98], and consequently does not generate fibrin clots directly, MASP-1-mediated fibrinogenolysis nevertheless releases the chemotactic fibrinopeptide B, which recruits cellular immune effectors without the pleiotropic effects of the proinflammatory anaphylatoxins released by complement [100]. In addition, MASP-1 can activate PAR4 on endothelial cells, leading to a distinct proinflammatory phenotype that favors recruitment of neutrophils over monocytes at the site of infection [101, 102]. Furthermore, MASP-1, and to a lower extent MASP-2, can proteolyze circulating high-molecular-weight kininogen (HK) and release bradykinin (BK) [103], which, in turn, strengthens proinflammatory endothelial activation and enhances vascular permeability.

Downstream complement effectors also induce proinflammatory and prothrombotic responses in vascular cells, largely amplifying transcription of prothrombotic factors with concomitant downregulation of clotting inhibitors. Since the most explosive clotting reactions are initiated by TF, amplification of the complement cascade at sites of microbial injury is supplemented by TF transcription in monocytes [104], neutrophils [105], and possibly platelets [106] and endothelium [107]. Complement induction of TF is primarily mediated by the potent C3a and C5a anaphylatoxins, which, either alone or in conjunction with Toll-like receptors (TLRs) [108], activate multiple downstream signaling events leading to activation of the NF-kB pathway and subsequent cytokine and TF production. Complement-induced proinflammatory cytokines (TNF-α, IL-6, IL-1β, CD40L, and others) can, in turn, reinforce TF transcription in vascular cells [109]. Once expressed on vascular cell surface, procoagulant activity is further enhanced through TF decryption. Both upstream, C3 [110], and downstream complement effectors, TCC complexes [111], support TF decryption through either surface exposure of anionic phospholipids or protein disulfide isomerase (PDI)-mediated TF oxidation [112].

In addition to TF activation, complement effectors modulate the thromboresistant phenotype of vascular endothelium [113]. Endothelial regulators control the initiation, amplification, and termination phases of the clotting cascade: endothelial TFPI is the main regulator of TF-initiated blood clotting [114]; ATIII adsorbed on surface heparan sulfate proteoglycans (HS-PGs) inhibits the amplification phase of coagulation [95], while endothelial TM initiates thrombin-dependent activation of protein C and regulates the termination phase of blood clotting [115]. TM is also a negative regulator of complement activation on endothelial surface [116]. In response to vascular injury, the endothelium is activated either directly, by microbial PAMPs binding to pattern recognition receptors (TLRs), or indirectly, through immune (complement), inflammatory (cytokines), or hemostatic (FVIIa, FXa, FIIa) mediators. Complement supports endothelial activation through multiple C1q, C3b (CR1 and CR3), and anaphylatoxin (C3a and C5a) receptors [117]. Complement induces shedding of anticoagulant regulators (HS-PGs, TFPI, and TM) from the endothelial surface [115, 118, 119], the release of procoagulant von Willebrand factor from Weibel-Palade bodies [120], and the rapid exposure of the leukocyte adhesion molecule P-selectin [120], thus promoting a prothrombotic environment. Furthermore, anaphylatoxin receptors initiate the transcriptional activation of a proinflammatory response with progressive expression of cell adhesion molecules (E-selectin, ICAM-1, VCAM-1), cytokines/chemokines (IL-1β, IL6, IL8, RANTES), and related receptors (VEGFC, IL-18R) [121, 122]. Similarly, endothelial activation by sublytic concentrations of C5b-9 MAC or the soluble inactive TCC com-

plexes induces differential expression of adhesion molecules supportive of recruitment and extravasation of leukocytes [123] and exposes anionic phospholipids with subsequent acceleration of clotting reactions and release of prothrombotic microparticles [124, 125].

Platelets are cellular entities situated at the interface between coagulation, inflammation, and immunity, with critical roles in hemostasis, immune surveillance, and the host response to microbial invasion [126]. Platelets activated by vascular trauma rapidly expose adhesion molecules, which enable their localization at injury sites, and release prothrombotic, proinflammatory, and antimicrobial mediators [127, 128]. A bidirectional communication between activated platelets and vascular proteolytic cascades exists, where platelets are activated by and in turn amplify the complement, coagulation, and kinin-kallikrein cascades. During infection, platelets promote recruitment of immune cells at sites of injury [129, 130], modulate endothelial permeability and subsequent leukocyte extravasation [131], and enhance antimicrobial functions of neutrophils [132]. Additionally, platelets mediate systemic responses through degranulation and release of bioactive inflammatory molecules and microparticles [133, 134]. Pathogens could activate platelets directly, through TLR recognition of microbial PAMPs [135, 136], or indirectly, following the engagement of complement C1q, C3b, and anaphylatoxin receptors [137] and/or the deposition of terminal complement complex C5b-9 on platelet surface [138, 139]. In return, platelets amplify complement deposition through the alternative [140] and/or classical initiation pathways [141], supporting a cross-activation/amplification step. Platelet degranulation releases complement factors locally [142], while platelet microparticles carrying complement receptors [143] have the potential to propagate complement activation systemically. P-selectin is a molecular convergence node for the innate immune, coagulation, and inflammatory responses on activated platelets deposited at sites of microbial or hemostatic insults. P-selectin is a C3b opsonin receptor on the activated platelet surface and amplifies complement activation through the alternative pathway [140]. In addition, P-selectin promotes leukocyte recruitment [144] and induces monocyte TF expression [145], phosphatidylserine exposure, and subsequent prothrombotic activity on the recruited monocytes [146]. Likewise, P-selectin helps capture circulating monocyte-derived TF microparticles [147], further concentrating clotting initiators at sites of injury and promoting thrombus growth. Consequently, it is not totally surprising that complement factor deficiency impairs platelet deposition and thrombus growth during the initial hemostatic response after experimental damage to the vessel wall [148].

The complement-coagulation crosstalk is bidirectional, and complement activation is amplified in prothrombotic environments (Fig. 12.4). Coagulation proteases have broad specificities and could activate complement factors. For example, FXIIa, the first coagulation protease of the contact clotting pathway, can also initiate the classical complement cascade through proteolytic activation of C1r/s proteases [149]. Thrombin has also been shown to proteolyze C3 and C5 in experimental murine models, producing bioactive C3a and C5a anaphylatoxins [39, 150]. Nevertheless, pure activation of downstream clotting proteases on synthetic phospholipid surfaces in nonhuman primates does not support C3 or C5 proteolysis [151], indicating that confounding variables may have contributed to thrombin-mediated complement activation in previous models. Thrombin stimulation of platelets at sites of vascular injury could indirectly promote complement activation through exposure of P-selectin [140], as mentioned above, or the release of chondroitin sulfate [152]. In addition to proteolytic crosstalk, the coagulation and complement pathways share common regulators. C1-INH is the main soluble inhibitor of both clotting factor FXIIa, kallikrein, and complement initiating proteases C1r/s and MASP-1/2 [74]. To a lower extent, antithrombin III, the main thrombin inhibitor, also inhibits MASP-1/2 [96]. On undisturbed endothelium, surface-exposed TM and HS-PGs act as negative regulators of both clotting reactions and complement activation/propagation [153, 154]. TAFI, a carboxypepti-

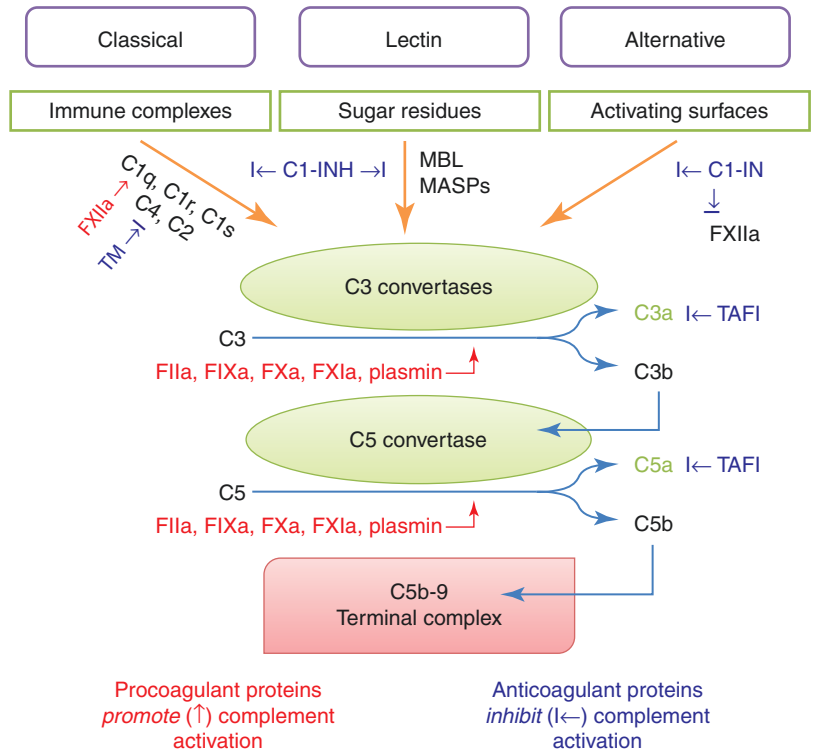


Fig. 12.4 Effects of the coagulation proteins on the complement pathways

dase that modifies fibrin making it less susceptible to plasmin proteolysis also desarginates C3a and C5a [155] and reduces their proinflammatory effects. While the physiologic role of coagulation-induced complement activation is not always clear, complement activation and generation of TCC intermediates are necessary for an optimal hemostatic response since both C3- and C6-deficient mice exhibit prolonged tail bleeding times [148, 156].

Prothrombotic Manifestations of Complement Deficiencies

The synergistic activities of complement, coagulation, and inflammation are required for an optimal and balanced hematologic response to vascular trauma. An inadequate response due to either the inefficient activation or, conversely, the improper regulation of the effector pathways may prove deadly for the host organism. Human pathologies associated with complement deficiencies highlight its essential roles in the innate and adaptive immunity [3, 6]. Deficiencies of factors from the three complement initiation pathways are associated with both an increased risk of recurrent infections, mainly bacterial, and the development of autoimmune disorders with a strong inflammatory component such as systemic lupus erythematosus (SLE) or SLE-like pathologies [157]. In contrast, deficiencies of terminal complement pathway components strongly associate with recurrent Gram-negative bacterial infections, due to impaired antimicrobial function [158], but not with autoimmune diseases, highlighting the role of complement opsonins in immune homeostasis. In general, deficiencies of complement effectors express phenotypically as autosomal recessive [157] and, due to intrinsic redundancy of immune responses, are compatible with life. However, the enhanced susceptibility to microbial infections increases the risk of systemic spreading and sepsis, with subsequent prothrombotic manifestations such as disseminated intravascular coagulation (DIC) [159].

Deficiencies of complement regulatory proteins can impair the hematologic response through

excessive activation of complement and/or hemostatic pathways. Deficiency of C1-INH, the main inhibitor of classical and lectin initiating proteases, leads to hereditary angioedema, an autosomal dominant pathology characterized by extravascular edema due to excessive production of bradykinin [160]. While the pathophysiology of the angioedema is probably complement independent and due to excessive activation of contact coagulation pathway and/or kinin-kallikrein system also controlled by C1-INH, low levels of C2 and C4 have been detected in these patients, suggesting unbalanced complement activation [158].

Deficiencies of C3 convertase regulators result in excessive deposition of C3b and C4b complement opsonins on self-structures. Primary C4BP deficiency is rare and results in atypical Behçet's disease complicated by angioedema [161]. C4BP polymorphisms associate with atypical hemolytic syndrome [162] and, surprisingly, represent a risk factor for venous thrombosis independent of its anticoagulant partner, protein S [163]. Impaired regulation of the alternative C3 convertase due to deficiencies in FH, FI, and/or MCP [164] leads to multiple pathophysiologies such as SLE-like autoimmune diseases, atypical hemolytic uremic syndrome (aHUS), and membranoproliferative glomerulonephritis (MPGNs) [165–167]. The uncontrolled AP C3 convertase depletes plasma C3 and promotes exacerbated C3b deposition primarily in kidneys and subsequent thromboinflammatory responses resulting in glomerular thrombotic microangiopathy, thrombocytopenia, and microangiopathic hemolytic anemia. Pharmacologic inhibition of complement effectors, such as eculizumab blockade of C5 activation [168], reduced the thrombotic burden in aHUS [169] and MGPN patients [170], indicating that hemostatic imbalance develops downstream of complement activation in these pathologies.

Similarly, deficiencies of membrane-bound complement regulators may expose host cells to the damaging effects of complement activation. DAF (CD55) and CD59 are glycophosphatidylinositol (GPI)-anchored cell surface negative regulators of complement convertases and the C5b-9 TCC, respectively. Mutations in the PIG-A gene that encodes the GPI-anchoring enzyme lead to the development of paroxysmal nocturnal hemoglobinuria (PNH), a complement-mediated disease characterized by the absence of CD55/CD59 on vascular cells [171]. The absence of these protective mechanisms allows AP-initiated complement cascade to progress on the surface of vascular cells resulting in intravascular hemolysis [168, 171]. Thrombophilia is a common clinical manifestation associated with PNH, with thromboembolism being the leading cause of mortality in PNH patients [172]. While the prothrombotic mechanisms are not completely understood, eculizumab inhibition of downstream complement effectors also reduces the incidence of thrombotic complications in PNH [173].

Complement-Coagulation Interplay in the Pathophysiology of Sepsis

Sepsis is an acute, progressive, systemic inflammatory response syndrome (SIRS) induced by an impaired hematologic response to infection [159, 174, 175] and a frequent complication of trauma. Severe sepsis and trauma complicated by multiple organ dysfunction syndrome (MODS) are leading causes of death in intensive therapy units worldwide [176]. Sepsis is initiated by the detection of pathogenic PAMPs within the vasculature, which trigger complement, coagulation, and inflammatory responses (Fig. 12.2). The excessive concomitant activation of multiple hematologic systems overcomes the regulatory networks in place and damages the host's own tissues and organs, ultimately leading to multiple organ failure and death [159]. In addition, survivors can exhibit sepsis-induced long-term sequelae such as diffuse pulmonary fibrosis [177] and cognitive impairment due to structural brain alterations [178], which affect their quality of life and long-term mortality [179]. Despite extensive research efforts, the pathophysiology of sepsis is not completely understood, and no treatments are currently available for sepsis. Experimental inhibition of inflammatory, coagulation, and complement cascade effectors have proven unsuccessful in sepsis trials [175], and as a result

new approaches are needed to characterize the systemic hematologic response and identify interacting nodes that control multiple pathways.

During initial stages of sepsis, complement activation is critical for the immune response to invading pathogens [180]. Complement activation can occur through all three initiating pathways, leading to C3 activation and formation of the terminal C5b-9 complex [181, 182]. Inhibition of complement activation at this stage negatively affects the outcome in sepsis due to impaired microbial clearance. Complement also modulates inflammatory and prothrombotic responses in sepsis. Complement anaphylatoxins are the main complement-induced inflammatory mediators as detailed above, affecting the permeability of blood vessels, leukocyte recruitment and activation, cytokine/chemokine expression, as well as TF expression on monocytes and endothelium [66, 183, 184]. The exacerbated activation of the complement cascade however, reflected by the increased plasma levels of C3a, C4a, and C5a anaphylatoxins [185], has been associated with multiple organ failure and enhanced mortality in septic patients [183]. In contrast, delayed complement inhibition ameliorates physiologic parameters, attenuates sepsis-induced inflammation, reduces thrombotic burden, and protects against multiple organ failure in a nonhuman primate models [181, 186].

Sepsis activates coagulation mainly through the TF-dependent pathway [187], although systemic activation of the contact pathway also occurs in patients and experimental models [188]. Coagulation activation is tightly interconnected with inflammation [189] and promotes DIC, the most important marker of poor prognosis in sepsis [159]. A prolonged thrombophilic environment is maintained through transcriptional activation of TF in vascular cells [190, 191] and depression of negative regulatory mechanisms, such as TFPI, APC, TM, and ATIII [189, 192, 193]. Complement contributes to sepsis-associated thrombophilia through multiple mechanisms. For example, C5a-C5aR signaling induces neutrophil TF expression [105] and procoagulant and proinflammatory cytokines that support monocyte TF expression [183], complement opsonins and TCC complexes support TF decryption [110, 111], while complement opsonins, anaphylatoxins, and TCC promote platelet activation and subsequent exposure of prothrombotic anionic phospholipid surfaces [194, 195]. Inhibition of complement effectors using compstatin, a C3 convertase inhibitor [181], or C5 convertase inhibitors [38, 69, 186], reduced thrombosis, inflammation, and associated organ injury in experimental septic models. These studies reveal the clinical importance of the complement-coagulation interplay during the development of sepsis and provide proof-of-concept strategies for concurrent therapeutic control of multiple hematologic pathways in sepsis.

Conclusions

Extensive studies have highlighted the intimate meshing between complement and coagulation responses during the pathophysiology of sepsis and trauma. The multifaceted crosstalk between the two pathways may lead to their reciprocal amplification creating a vicious cycle that contributes to multiple organ failure (MOF) and death. Since activation of the complement system occurs early during organ failure progression, use of complement inhibitors to prevent MOF is a logical approach. The complement therapeutics under development or in clinical use include: (i) enhancing the expression of negative regulators (CD55, CD59, soluble complement receptor-1) and supplementing the natural complement inhibitors (C1 inhibitor); (ii) blocking antibodies, peptides, aptamers, or small inhibitory molecules against C3 and C5 convertases; and (iii) antibodies that neutralize complement-derived anaphylatoxins (C3a and C5a) or inhibit their cognate receptors. Complement inhibition, however, may compromise the host defense against pathogens, resulting in increased risk of secondary infections, or the clearance of damaged cells and structures, resulting in impaired immune homeostasis and faulty healing. Therefore, identification of appropriate therapeutic windows and development of strategies to prevent exacerbated complement activation while preserving its protective and reparative functions are important challenges that need to be addressed.

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13

Neutrophils, Inflammation, and Innate Immunity in Trauma-Induced Coagulopathy

Christopher D. Barrett and Michael B. Yaffe

Introduction

It should come as no surprise that, as we learn more about the innate immune and coagulation systems, we continue to discover additional ways that the two are intimately and inseparably linked [1]. Reducing it to the simplest terms, one can imagine that it would be biologically advantageous to have a hemostatic, anti-microbial (inflammatory), pro-regenerative response following traumatic injury. Of particular interest in mediating cross-talk between the coagulation and immune systems is the polymorphonuclear leukocyte (PMN), or neutrophil, so named for its neutral staining characteristics on Wright Stain compared to the other two granulocyte populations (basophils and eosinophils) [2]. Neutrophil antibacterial properties have been known to surgeons for two and a half centuries, where during the 7 Years' War in 1761 the British surgeon John Hunter noted a delay in blood spoilage when the "buff" colored layer from one blood sample was added to another blood sample [3]. Centuries later this innate immune cell, with its respiratory burst (via the NADPH oxidase complex) and toxic protein granule products, is now known to do far more than just provide antibacterial defenses. In the modern era, neutrophils have been shown to play a key physiologic role in response to tissue damage and wound healing, coagulation, and endothelial barrier function and injury [4–12]. Unfortunately, loss of tight control of neutrophil function can also have serious pathophysiologic consequences, with aberrantly activated neutrophils contributing to diseases such as the acute respiratory distress syndrome (ARDS), transfusion-related acute lung injury (TRALI), ischemia-reperfusion injury, sepsis, and more recently the coagulopathy associated with traumatic injury [12–17].

Following traumatic injury, areas of tissue damage and the associated injured vascular endothelium lead to exposure of the extracellular matrix (ECM) and a shift in function and expression of coagulation regulatory molecules on the endothelium (reviewed elsewhere in this text). Additionally, injured endothelial cells and the Type I and Type II activation of surrounding un-injured endothelial cells in response to inflammatory chemokines/cytokines and ischemia-reperfusion lead to increased expression of cell-surface adhesion molecules [15, 18–20]. The exposed sub-endothelium and upregulated endothelial adhesion molecules, including P-selectin, E-selectin, ICAM-1, and

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VCAM-1, act to recruit neutrophils, platelets, and other leukocytes (e.g. macrophages) to the site of injury, in order to provide antibacterial defenses, promote tissue remodeling, and release tethered growth factors and cytokines/chemokines that promote inflammatory responses, wound healing, and hemostasis [4, 5, 21–26]. In the case of traumatic coagulopathy, the associated severe injury results in the release of large amounts of inflammatory chemo/cytokines and damage-associated molecular patterns (DAMPs), which causes priming and systemic activation of neutrophils. In an indiscriminate fashion, these activated neutrophils release significant amounts of extracellular ROS via the NADPH-oxidase complex, release an array of toxic and proteolytic enzymes via degranulation, and extrude their DNA contents decorated in histones and granule proteins to effect further endothelial injury, propagate coagulation through increased thrombin generation and decreased anticoagulant protein activity, alter fibrinolysis, and cause end-organ damage.

Neutrophil Localization in Injury and Inflammation

Neutrophils exist in two populations outside the bone marrow, the marginated pool and the circulating pool, each comprising about half of the total peripheral blood PMNs [27]. Neutrophils within the circulation interact with the endothelium via the leukocyte adhesion cascade in the post-capillary and collecting venules primarily via PSGL-1 and integrins (αβ-heterodimers), although other proteins and ligands like L-selectin, CD44, and ESL-1 likely also play a role [21]. In homeostatic conditions, neutrophils will tether and roll along the endothelium in a selectin-ligand-dependent manner (the main neutrophil ligand being PSGL-1), with very high association and dissociation constants, but will not become adherent or transmigrate across the endothelial barrier [28]. Upon stimulation by increased selectin-mediated signaling, chemokine activation of G-protein coupled receptors, and increased inflammatory cytokines and chemotactic factors, there is a change in conformation of integrins (LFA-1, VLA-4) on the cell surface from a bent low-affinity to an extended low-affinity state, followed by transition to a high-affinity extended conformation for their ligands, primarily ICAM-1 and VCAM-1 [28–31]. The final transition of these integrins to their extended high-affinity state may, at least in part, be dependent on shear stress via a "catch bond" phenomena [32]. This binding interaction of the PMN integrins with their endothelial ligands leads to the arrest of rolling and tight adhesion of the neutrophil to the endothelial surface, whereupon the neutrophil can transmigrate across the endothelial barrier in response to further chemotactic stimuli in a paracellular or transcellular manner. The initial migration out of the circulation is then followed by subsequent chemotaxis into the underlying extravascular tissue [28, 33–35]. This process of neutrophil adhesion and transmigration across the endothelial barrier is thought to contribute to microvascular hyperpermeability under certain circumstances [36]. Typically, PMN transmigration is considered to be a polarized unidirectional process with very few neutrophils returning to the circulating granulocyte pool. In mice, however, it has been shown that reverse transmigration back into circulation can potentially turn localized inflammation into systemic inflammation with secondary organ damage [37], although to date it is unknown if human PMNs exhibit this same phenomenon.

In the case of traumatic injury with a denuded endothelium and exposed subendothelial matrix, neutrophil localization is primarily platelet-dependent, with a similar process of transmigration occurring when neutrophils adhere to activated adherent platelets [38]. Platelets bind at the site of exposed ECM in a vWF/GPIb-dependent manner and recruit PMNs following platelet activation, initially via P-selectin/PSGL-1 binding and subsequently by binding of the neutrophil β_2 integrin Mac-1 directly to platelet GPIb and possibly also to fibrin [39] that is adherent to platelet $\alpha IIb\beta_3$ integrin (GPIIbIIIa) within the developing thrombus [38, 40–48]. If the injury is severe enough (e.g., severe traumatic injury or sepsis), however, the ability of neutrophils to localize via adhesion and transmigration

becomes impaired by the overwhelming inflammatory signaling response. Neutrophils become "paralyzed" in such states, not only rendering them unable to tightly adhere in a spatially localized manner, but also unable to chemotax toward sites of infection and injury with increased membrane rigidity and capillary sequestration [33, 49]. Recent studies have shown that traumatic injury causes the release of distinct human damage-associated molecular patterns, or DAMPs, including mitochondrial formyl-peptides and mitochondrial DNA, whose release can activate the same G-protein-coupled formyl peptide receptor-1 (FPR1) and TLR9 as their bacterial formyl-peptide and DNA counterparts, with FPR1 and TLR9 being expressed on both neutrophils and endothelial cells [50–54]. These DAMPs are likely responsible for the clinical similarities of shock and inflammation following trauma and sepsis, including the systemic neutrophil responses. In these states of critical illness there is reduced adhesion molecule expression secondary to the upregulation of iNOS, HO-1, and PPARγ, early priming and systemic activation of neutrophils that is followed later by desensitization to future priming and activation from prolonged stimulus exposure, and CXCR2 downregulation by TLR2 and TLR4 agonism [33, 55]. Thus, states of critical illness, inclusive of significant trauma, have the potential to lead to high levels of non-localized systemic neutrophil activation with subsequent innate immune compromise secondary to paralysis and neutrophil desensitization.

Neutrophil NADPH-Oxidase Priming and Highly Reactive Oxygen Species Production

One of the most important tools available to neutrophils following traumatic or infectious insults to the host is their ability to produce highly reactive oxygen species (ROS) via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. At rest, the NADPH oxidase components are not fully assembled, with gp91phox and p22phox making up a membrane protein called flavocytochrome b_{558}, a transmembrane αβ-heterodimer with 2 non-identical coordinated heme prosthetic groups that make up the electron transfer system across the membrane to produce superoxide anion via electron transfer to O_2 [56, 57]. The other proteins p40phox, p47phox, p67phox, and Rac-2 GTPase remain in the cytosol away from the flavocytochrome b_{558} complex and exist in several uncomplexed and partially complexed states [58]. Following the stimulation of neutrophils with an agonist such as LPS, TNF-α, PAF, or fMLP (amongst many others) the neutrophil becomes "primed" for subsequent activation of the NADPH oxidase complex in response to a second stimulus [59–62]. The key feature of priming is that the response primed neutrophils have to a given second stimulus is exponentially greater than that of unprimed neutrophils to the same stimulus, lending a critical mechanism of regulation for when and under what conditions neutrophils generate ROS. Additionally, different priming agents lead to proportionally different amounts of extracellular versus intracellular ROS by the PMN NADPH oxidase complex, where, for example, the priming agents PAF and IL-8 lead to more extracellular ROS after a second stimulus than priming by TNF-α [63].

On a molecular level, priming occurs via multiple mechanisms and depends on the priming agent, but the two most commonly implicated mechanisms involve (a) p47phox phosphorylation with subsequent conformational changes that allow for additional phosphorylation and binding site exposure to facilitate both the binding of p47phox to the p40phox:p67phox complex as well as later binding to flavocytochrome b_{558} at the plasma membrane during activation, and (b) direct mobilization of specific and gelatinase granules containing flavocytochrome b_{558} to the cell membrane to increase the number of potential NADPH oxidase complexes that can be assembled [58, 64]. Upon exposure to a second stimulus, the primed neutrophil now assembles the holoenzyme NADPH oxidase complexes and proceeds to generate a large amount of superoxide anion at the plasma membrane and within phagosomes that leads to several toxic ROS species, a

process that can lead to extensive tissue damage and endothelial cell activation, injury and cell death [65–67]. Importantly, these priming and subsequent activation events are the molecular explanation underlying the classic "two hit hypothesis" for multiple organ failure after trauma [68].

The underlying signaling pathways for priming involve several key mediators which also depend on the priming agent. Brown and colleagues [69] showed that TNF-α priming of human neutrophils for subsequent respiratory burst activation was strictly dependent on p38 MAPK phosphorylation of p47phox and p67phox, whereas PAF priming was independent of p38 MAPK activity, and resulted in only p67phox phosphorylation, with Sheppard et al. subsequently finding that PAF priming led to p67phox translocation to the plasma membrane [70]. Additionally, Brown et al. showed that regardless of the priming agent, fMLP activation for ROS production by primed neutrophils was reduced by 70% when p38 MAPK was inhibited using the specific p38 MAPK small molecule inhibitor SB203580, whereas complement-opsonized zymosan (OPZ) activation of primed neutrophils was independent of p38 MAPK and instead appeared to involve the Erk1/2 MAPK pathway. Subsequent elegant work by Dang et al. [71] using human PMN showed that Ser345 on p47phox, which lies within a MAPK consensus sequence (-PXSP-), is the phosphorylation target for priming by TNF-α via p38 MAPK activation and by GM-CSF via Erk1/2 activation, with both kinase pathways converging on phosphorylation of Ser345. Addition of a direct p38 MAPK inhibitor (SB203580) or indirect Erk1/2 inhibitors (PD98059 and UO126 for inhibition of MEK1/2 upstream) abrogated priming by TNF-α and GM-CSF, respectively. Further, evidence for in-vivo relevance was observed through increased ROS production in response to fMLP along with increases in phospho-Ser345-p47phox, phospho-p38 MAPK, and phospho-Erk1/2 in synovial PMN from human rheumatoid arthritis (RA) patients compared to circulating PMN from the same RA patients and healthy human controls, and competitive substrate assays using cell-permeable TAT-Ser345 showed inhibition of both basal and fMLP-stimulated ROS production of these PMN [71].

The importance of phosphorylation at Ser345 on p47phox for human neutrophil priming by TNF-α was further clarified by El-Benna and colleagues, who showed that the *cis-trans* prolyl isomerase Pin1 binds to phospho-Ser345 form of p47phox and induces conformational changes that allow for the additional required serine phosphorylations by PKC to prime PMN [72], as well as facilitate binding of p47phox to the other cytoplasmic subunits p40phox and p67phox. It is thought that these conformational changes and additional phosphorylation events lead to exposure of cryptic SH3 domains in p47phox that in non-primed (non-phosphorylated) states remain bound to a proline-rich autoinhibitory region within p47phox itself. Thus, phosphorylation of p47phox liberates the SH3 domains to bind the proline-rich region of the cytosolic tail on the p22phox component of the membrane-bound cytochrome, and provides a targeting mechanism for p47phox and its associated cytosolic oxidase components to bind at the membrane for holoenzyme assembly during activation. Other signaling pathways that prime and activate the NADPH oxidase complex result in p47phox phosphorylation on different serine residues, such as Akt phosphorylation of Ser304 and Ser328 in response to fMLP, IL-8, and GRO-α activation of PI3K by binding their respective GPCRs [73–75]. Whether or not priming via phosphorylation of cytosolic oxidase components leads to pre-assembly of the holoenzyme NADPH oxidase complex at the plasma membrane is a topic of debate, and it is likely that this may be the case with some priming agents but not with others. Yaffe and colleagues have shown using membrane fractionation techniques that priming of human neutrophils with TNF-α does not lead to translocation of p47phox or p67phox to the plasma membrane despite causing phosphorylation of both, while stimulation with the non-physiologic direct PKC activator phorbol myristate acetate (PMA) does cause membrane translocation of p47phox and p67phox. This observation argues against a role for oxidase pre-

assembly at the plasma membrane in p38 MAPK-dependent TNF-α priming of human neutrophils. Others have shown that LPS-induced priming of human PMN is a p38 MAPK-dependent process resulting from cytochrome b_{558} shuttling to the plasma membrane via specific granules along with limited p47phox translocation, while Sheppard et al. showed that priming by PAF resulted in p67phox membrane translocation [70, 76, 77].

With respect to the PMN NADPH oxidase complex and trauma-induced coagulopathy, direct evidence for a role of neutrophil-derived ROS in traumatic coagulopathy is limited to date, but there are numerous studies that suggest this is the case and a recent study published by our group supports that PMN ROS is involved [63]. In a study by Jacobi et al. it was shown that human PMNs from hemodialysis patients exist in a highly primed state and that co-culture with human umbilical vein endothelial cells (HUVEC) leads to significant amounts of HUVEC apoptosis, tissue factor and IL-8 production, and immediate increases in HUVEC cell surface P-selectin compared to healthy human controls [65]. Importantly, in those experiments a membrane barrier was used to prevent direct PMN-HUVEC adhesion, and further showed that the observed effects on HUVEC were abrogated by the addition of superoxide dismutase (SOD) and catalase. These observations directly implicated neutrophil-derived ROS and other soluble products of primed and activated PMN in causing endothelial cell activation and pro-coagulant states in addition to mediating endothelial cell injury and death. These findings are supported by earlier studies that showed rat pulmonary artery endothelial cell injury and death in response to activated PMN could be prevented with early addition of catalase and at later time points showed significant synergistic endothelial cell killing by PMN-derived ROS and proteases [66]. More recently, neutrophils from human polytrauma patients were shown to cause significant amounts of endothelial progenitor cell (EPC) killing compared to healthy human controls that could be prevented by pre-treatment of PMN with the NADPH oxidase inhibitor diphenyliodonium chloride. In contrast, however, the inhibition of neutrophil elastase did not show any protective effect [78]. Given the important role of EPCs in tissue and vascular repair following traumatic injury, it is thought that the excessive neutrophil priming and activation seen in severe traumatic injury may prevent wound healing and vascular repair through killing of EPCs. Additionally, it is known that formation of neutrophil extracellular traps (NETs) requires ROS generation by the NADPH oxidase complex and that NETs propagate coagulation through thrombin generation, cleavage of TFPI, and direct endothelial damage (discussed in more detail below) [6, 7, 9, 79]. The above findings are supported by recent work from our group, where we found a robust priming of human neutrophils by human serum (i.e., the liquid product of clotted whole blood) and clotted plasma for ROS production in response to formylated peptides, an in-vitro approximation of what neutrophils may see following traumatic injury with blood clotting and release of human mitochondrial-derived formylated peptides (prototypic DAMPs) [63]. We found that this phenomenon was dependent on the alternative pathway of complement generating C5a during blood coagulation, and importantly that it preferentially primed PMN for early extracellular ROS generation that could degrade HUVEC barriers. We then evaluated plasma from trauma patients in hemorrhagic shock, where the coagulation system has been activated, and similarly found that their plasma had elevated C5a levels that were dependent on the alternative pathway, and that this trauma plasma C5a primed naive neutrophils for predominantly extracellular ROS generation. In the same study we then used a mouse model of trauma hemorrhage known to generate end-organ injury and a protein C-mediated traumatic coagulopathy (previously described [80, 81]), comparing wild type and sham mice with p47phox KO mice whose PMNs are incapable of generating ROS via the NADPH oxidase complex. We found that the p47phox KO mice were protected from both end-organ (lung) injury and protein C–mediated traumatic coagulopathy, directly implicating neutrophil ROS in the pathogenesis of trauma-induced coagulopathy.

Neutrophil Granule Proteins and Coagulation

Another key component of neutrophil effector responses to inflammatory stimuli and injury is the release of enzymes and proteases from neutrophil granules. These enzymes and proteases are likely to be direct contributors to the coagulopathic state, further propagating the deleterious feed-forward cycle of traumatic coagulopathy and inflammation. Many of the same stimuli responsible for neutrophil priming and ROS generation also lead to degranulation of primary (azurophilic), secondary (specific), and tertiary (gelatinase) granules and secretory vesicles in a stimulus-dependent fashion, with more potent stimuli being required for primary granule release given their more toxic contents and less potent stimuli required for the sequentially less toxic granule release (Tables 13.1, 13.2, and 13.3) [82, 83]. The function of granule products varies widely, including catalyzing HOCl production from ROS (myeloperoxidase/MPO), regulation of the coagulation and fibrinolysis systems (elastase, cathepsin G), direct microbicidal activity (defensins, lysozyme), ECM remodeling via proteolysis and cytokine/chemokine processing (matrix metalloproteinases/MMPs, elastase, other serine proteases), and neutrophil priming via specific and gelatinase granule secretion (flavocytochrome b_{558}) [84, 85].

A critical discovery was the observation that secretion of neutrophil elastase and cathepsin G from azurophilic granules leads to cleavage of tissue factor pathway inhibitor (TFPI) and subsequent tissuefactor (TF), and factor XII-dependent activation of coagulation [6]. In this study by Massberg et al., they observed in-vivo that fibrin deposition following vascular injury was significantly reduced in mice deficient in neutrophil elastase and cathepsin G due to deficient cleavage of TFPI and that the process of TFPI cleavage by neutrophil serine proteases in WT animals was dependent on NETs to co-localize these proteases with TFPI. Treatment of WT mice with H2A-H2B-DNA-specific antibody suppressed coagulation and fibrin formation but did not lead to additional reduction in fibrin formation in the elastase −/−, cathepsin G −/− mice. Further, they showed in-vitro that

Table 13.1 Neutrophil agonists that regulate chemotaxis, priming, and/or activation in either solution phase or adherent neutrophils

Agent	Chemotaxis	Priming	Activation (ROS production, degranulation)
N-Formyl peptides (fMLP)	+	+	+
Mitochondrial DNA	+	+	+
PAF	+	+	+
PF4	+	+	
FPB	+		
LTB_4	+	+	+
Gro-α	+	+	+
IgG/IgM			+
LPS		+	
C5a	+	+	
G-CSF	+	+	+
GM-CSF	+	+	
TNF-α	+	+	+
IFN-γ		+	
IL-1β	+	+	+
IL-6	+		
IL-8	+	+	+

Adapted from references [39, 50, 59–63, 83, 137–147]

Table 13.2 Intramembranous proteins found within neutrophil granules and secretory vesicles

Azurophilic (primary) granules	Specific (secondary) granules	Gelatinase (tertiary) granules	Secretory vesicles
Membrane	Membrane	Membrane	Membrane
Granulophysin (CD63)	MAC-1 (CD11b/CD18)	MAC-1 (CD11b/CD18)	MAC-1 (CD11b/CD18)
CD68	Cytochrome b_{558}	Cytochrome b_{558}	LFA-1 (CD11a/CD18)
V-type H^+-ATPase	TNF-R	DAG lipase	Cytochrome b_{558}
	fMLP-R	fMLP-R	fMLP-R
	u-PA receptor	u-PA receptor	u-PA receptor
	SCAMP	SCAMP	SCAMP
	Laminin-R	Laminin-R	CR1
	Thrombospondin-R	V-type H^+-ATPase	C1q-R
	Vitronectin-R	VAMP-2	DAF
	Fibronectin-R		Alkaline phosphatase
	VAMP-2		VAMP-2
	G-$protein_{\alpha}$-subunit		CD14
	CD66		CD16
	CD67		V-type H^+-ATPase
	CD15 antigens		Neutral Endopeptidase (CD10)
	NB 1 antigen		Aminopeptidase N (CD13)
	Rap1		Tyrosine Phosphotase (CD45)
	Rap2		

Adapted from references [83, 148]

Table 13.3 Soluble proteins found within neutrophil granules and secretory vesicles

Azurophilic (primary) granules	Specific (secondary) granules	Gelatinase (tertiary) granules	Secretory vesicles
Matrix	Matrix	Matrix	Matrix
Myeloperoxidase	Collagenase (MMP-8)	Gelatinase	Tetranectin
Defensins	Gelatinase	Lysozyme	Other plasma proteins
Bactericidal permeability increasing protein	Lactoferrin	β_2-microglobulin	
Cathepsins	Lysozyme	Acetyltransferase	
Elastase	u-PA	Tetranectin	
Lysozyme	β_2-microglobulin		
Proteinase 3	hCAP-18		
Azurocidin (heparin-binding protein, CAP37)	Histaminase		
α_1-antitrypsin	Heparanase		
α-mannosidase	NGAL		
Acid β-glycerophosphatase	Sialidase		
Acid Mucopolysaccharide	SGP28		
β-Glycerophosphatase	B_{12}-binding protein		
β-Glucuronidase			
N-acetyl-β-Glucosaminidase			
Sialidase			
Ubiquitin-protein			

Adapted from references [83, 148]

DNase was also able to abrogate the increased TF activity and TFPI degradation by neutrophil serine proteases observed in reconstituted nucleosome coagulation assays. These findings showed in a substantial way that neutrophils are direct regulators of the natural anticoagulant system and that neutrophil dysfunction can have powerful consequences on coagulation and hemostasis.

In addition to proteolytic cleavage of the natural anticoagulant TFPI, it is known that neutrophil elastase, MMP-8, and MMP-9 can promote coagulation by cleaving proteoglycans in exposed blood vessel walls that model injured or denuded blood vessels such as those seen in trauma [86]. By degrading part of the proteoglycan matrix, vWF binding sites on vessel wall collagen are exposed and this leads to a dramatic increase in platelet adherence under flow conditions through vWF-GpIb binding interactions, which can further amplify neutrophil recruitment and activation. This pro-coagulant effect seen using formyl peptide-activated human neutrophils, PMN supernatants, and purified neutrophil elastase, MMP-8, and MMP9 could be completely mitigated by a combination of serine- and metalloprotease inhibitors. Beyond the pro-coagulant activity of neutrophil proteases in the subendothelial matrix, co-culture models with neutrophils and multiple different human endothelial cell lines have shown endothelial cell damage and killing by neutrophil elastase, with augmentation by ROS observed in many of them [10, 87, 88]. Neutrophil elastase and cathepsin G have shown a potent ability to cleave VE-cadherin and disrupt endothelial cell monolayers in-vitro, providing a possible mechanism for the vascular permeability and shock seen in trauma patients with severe inflammatory responses despite adequate resuscitation [11]. Another mechanism by which neutrophil elastase may contribute to vascular hyperpermeability and hypotension in traumatic injury is through the production of E-kinin from high-molecular-weight kininogen, which has been demonstrated in a rat model and is likely mediated by binding of the GPCR bradykinin β_2 receptor [89], and in humans a correlation between plasma levels of neutrophil elastase and pulmonary vascular permeability has been demonstrated in patients with severe pneumonia [90].

Beyond coagulation, neutrophil elastase also has a significant role in altering fibrinogen and the fibrinolysis pathway and is likely to be involved in the development of trauma-induced coagulopathies within the hyperfibrinolysis and fibrinolysis shutdown phenotypes. For example, elastase is capable of cleaving the Aα subunit of fibrinogen at the Val (Aα21)-Glu (Aα22) bond, thus removing the Aα17–20 peptide segment that classically forms the "knob" required for fibrin polymerization after thrombin cleavage at Aα16 [17, 91] and functionally reduces the amount of fibrinogen available to participate in clot formation. Elastase cleaves factor XIII in to a truncated and highly active form that crosslinks fibrin and may also tether α2-antiplasmin (A2AP) to fibrin, increasing clot resistance to fibrinolysis [92]. In contrast, elastase also cleaves plasminogen activator inhibitor-1 (PAI-1) and A2AP rendering both unable to inhibit tPA and plasmin, respectively, which promotes fibrinolysis [93, 94]. Elastase cleavage of plasminogen is also well described, leading to several different molecular species that have variable effects on fibrinolysis, and while the effects are generally considered pro-fibrinolytic this is likely nuanced and under certain conditions the opposite (resistance to fibrinolysis) may occur. When elastase cleaves plasminogen, mini-plasminogen (the C-terminal part of the protein containing the 5th Kringle domain and the protease domain) and angiostatin (the N-terminal part of the protein containing the Kringle 1–4 or Kringle 1–3 domains) are generated [95, 96]. Mini-plasminogen is more rapidly converted into mini-plasmin than plasminogen is to plasmin, and thus is better at promoting fibrinolysis [96]. However, the resulting angiostatin K1–4 is converted into angiostatin K1–3 by additional elastase cleavage and has a similar binding constant for fibrin as plasminogen does, suggesting that angiostatin may compete with plasminogen for fibrin binding sites and prevent plasmin formation [95]. There is some experimental data to support that once enough angiostatin K1–3 is generated and the plasminogen pool is depleted, the effect of elastase is one of resistance to fibri-

nolysis, and after prolonged co-incubations (e.g. 6 hours) of elastase with plasminogen prior to use in fibrinolysis assays there is a complete loss of fibrinolysis/fibrinolytic function [97]. Other experimental data suggests that elastase has direct proteolytic activity against polymerized fibrin [17, 98], although functional consequences of this interaction have not been consistently observed in the literature where others such as Wu et al. have not observed any measurable fibrinolytic effect of elastase on polymerized fibrin and thus its functional relevance remains a point of debate [93]. Ultimately the effects of PMN elastase on fibrinolysis are complex and context dependent, and are very likely involved in the development of traumatic coagulopathy. Our group is working toward generating a more complete description of the biochemical kinetics of all the known elastase targets in the fibrinolysis pathway to generate an ordinary differential equations model of how and when elastase generates different fibrinolysis phenotypes that also accounts for thrombin generation, which is likely a key component in determining the resultant fibrinolysis phenotype.

Another important granule protein, myeloperoxidase (MPO), is widely accepted as a critical effector enzyme for microbial killing through the production of hypochlorous acid from H_2O_2 and also has diverse effects on endothelial cell function and coagulation [99]. In human patients, serum MPO levels strongly correlate with endothelial dysfunction as measured by brachial artery dilation studies, and MPO is known to sequester vasoactive NO by using it to produce pro-inflammatory reactive nitrogen species [100, 101]. Support for MPO's direct vasoactive role is further demonstrated in MPO-deficient mice that exhibit resistance to endotoxin-mediated vasomotor dysfunction [102]. In-vitro studies have shown direct injury to endothelial cells by MPO-bearing neutrophilic microparticles, released upon neutrophil activation, with the loss of endothelial cell membrane integrity and concomitant morphologic changes consistent with endothelial damage, and others have shown direct endothelial cell cytolysis resulting from MPO-generated HOCl [103, 104]. NETs are coated with MPO, and the ability of NETs to cause direct endothelial cell cytotoxicity can be partially inhibited by the MPO inhibitor dihydrolipoic acid [9]. MPO is also capable of endothelial barrier transcytosis independent of neutrophil emigration in a heparin-dependent mechanism and has been implicated as a catalyst in ECM protein tyrosine nitration, including of fibrinogen and fibronectin, leading to more rapid fibrin clot formation and factor XIII-dependent fibrin crosslinking with a resulting pro-thrombotic state [105, 106]. MPO endothelial transcytosis lends further support for the ability of neutrophil activation to cause diffuse non-specific vascular inflammation and thrombosis, and the observed heparin dependence of MPO-endothelial cell interactions provides an explanation for the anti-inflammatory effects of heparin [106, 107]. Taken together, it is likely that MPO from activated neutrophils in traumatic injury causes direct, systemic endothelial and sub-endothelial damage, vasomotor dysfunction, and thrombosis that significantly contributes to trauma-induced coagulopathy.

Neutrophil Extracellular Traps: A Link Between Innate Immunity and Coagulation

In recent times, much interest has been directed toward the phenomenon of neutrophil extracellular traps, or NETs, where neutrophils extrude their nuclear DNA decorated with histones and toxic granule contents (e.g., myeloperoxidase, elastase, defensins, serine proteases) in a process termed NETosis [108]. It seems apparent that NETs are yet another mechanism by which neutrophils provide innate immunity, releasing a toxic web that can trap and kill bacteria [6, 109]. Additionally, NETs have now been shown to propagate coagulation and thrombus formation by generating thrombin in a platelet-dependent and platelet-independent manner, and facilitate tissue factor pathway inhibitor cleavage by serine proteases bound to NETs leading to TF and factor XII-dependent coagulation [6, 7]. NET formation is dependent on several key prerequisites, including ROS production, migration of granule

contents to the nucleus, and in certain circumstances require Mac-1 outside-in signaling [108, 110]. Stimulation of platelet TLR4 by bacterial LPS has been shown to rapidly induce NETosis via increased platelet binding to adherent neutrophils, and there are many other non-infectious agonists of TLR4 such as HMGB1 that are released in traumatic injury and have been implicated in NET formation [7, 109, 111]. It is not surprising, then, that NETs have been shown to occur in other non-infectious scenarios in both humans and mice (e.g., autoimmune vasculitis, TRALI, ventilator-associated lung injury), and there are numerous other stimuli for NETosis beyond TLR4 stimulation (e.g., ANCA antibody) [110, 112]. NETs form in the microvascular system such as liver sinusoids and pulmonary microvasculature where beyond capturing bacteria they can cause microvascular thrombosis and ischemia, lead to direct endothelial and epithelial damage, and cause end-organ injury, and there may also be a role for NETs in the pathogenesis of both arterial and venous thrombosis [8, 9, 108, 109]. As it relates to trauma, the role of NETs in the injury inflammatory response has been postulated based on the increased understanding of the role of NETs in disease, and just recently McIlroy et al. demonstrated in-vivo that NETs are formed in human patients following traumatic injury and subsequent surgery [113, 114]. Thus, following traumatic injury, intravascular NET formation is likely to participate in physiologic hemostasis, and loss of regulation of NETosis may be a major driver in tipping the balance towards pathologic coagulopathy.

Finally it is worth pointing out that in 2007, Brohi et al. [115] showed that one of the hallmarks of TIC following severe traumatic injury is an early increase in activated protein C (aPC) production due to tissue hypoperfusion, with later development of a prothrombotic state that likely develops secondary to exhaustion of the protein C anticoagulant pathway. This fits well with the concept of NETosis, whereby severe trauma leads to high levels of microvascular NET formation and subsequent endothelial damage, thrombus generation, and end-organ and tissue ischemia that may be responsible for the hypoperfusion-associated aPC generation observed by Brohi and colleagues. Furthermore, NETs also provide a procoagulant scaffold to facilitate the observed late thrombogenic state seen in patients who developed TIC. Given all the known and potential points of intersection between traumatic injury, NET formation, and coagulation it is all but certain that NETs play a major role in the pathogenesis of trauma-induced coagulopathy.

Early Activation of Innate Immunity in Trauma: Danger, Damage-Associated Molecular Patterns, and the Late Immunosuppression of Critical Illness

The innate immune system is the body's first-line defense against invading pathogens and other dangerous processes to the host such as tissue damage. In order to provide a rapid response to these threats, the innate immune system has evolved mechanisms to recognize common "danger" signals occurring in nature from *both* self and non-self, a theory originally championed by Polly Matzinger that has gained traction amongst many in the trauma community. This "danger signal" model may offer key insights into entities such as the systemic inflammatory response syndrome (SIRS or "sterile sepsis") often seen following severe trauma [50, 116, 117], where high levels of danger signals may be inadvertently released. Of particular interest in traumatic injury are the human damage-associated molecular patterns, or DAMPs, including mitochondrial formylated peptides and mitochondrial DNA (mtDNA), whose release is observed following traumatic injury and can activate the same G-protein-coupled formyl peptide receptor-1 (FPR1) and TLR9 as their bacterial formylated peptide and DNA counterparts [50]. It has been shown that FPR1 and TLR9 are expressed on both neutrophils and endothelial cells, which supports the notion that human DAMPs play a significant role in the similarities of shock and

inflammation observed following trauma and sepsis [50–54]. Human trauma and femur fracture patients have thousands-fold higher plasma mtDNA levels than healthy human controls, and mitochondrial products from trauma patients and femoral fracture reamings cause increased IL-8, MMP-8, and MMP-9 release in human PMN that can be inhibited by FPR1 blockade and additionally show potent neutrophil chemotactic activity [50, 118]. Mice rapidly develop peritonitis in response to mitochondrial DAMPs (MTD), while rat models consistently demonstrate end-organ neutrophil infiltration and pro-inflammatory cytokine production in the liver and lungs as well as significant increases in plasma concentrations of TNF-α, IL-6, and IL-10 in response to MTDs with the implication of the p38 MAPK, p44/42 MAPK, and NF-KB pathways through TLR9 and FPR1 signaling [50, 118, 119]. Importantly, these responses are absent in both nuclear DNA and vehicle controls. Trauma and hemorrhagic shock (T/HS) models of rats have provided additional evidence that significant amounts of mtDNA get released in states of shock, and there is some limited evidence that plasma mtDNA levels can help quantify inflammatory tissue injury and differentiate sepsis from sterile SIRS in non-human primates [120, 121].

Another important DAMP in neutrophil function under increasing investigation is the high-mobility group box 1 protein (HMGB1), originally thought to function only as a nuclear protein responsible for transcription regulation by bending DNA to facilitate binding of other regulatory protein complexes, including those from the nuclear hormone receptor family, V(D)J recombinases, and p53-p73 transcriptional complexes [122]. More recently, HMGB1 has been found to act as a prototypic DAMP when released into the extracellular compartment in response to cellular stress by immune cells, hepatocytes, platelets, and endothelial cells in addition to passive release by necrotic cells [123]. HMGB1 is now known to bind and activate the receptor for advanced glycation end products (RAGE) and several pattern recognition receptors including TLR2, TLR4, and TLR9 depending on the scenario, with HMGB1 levels being significantly elevated in human trauma patients [123, 124]. In a murine hemorrhagic shock/resuscitation (HS/R) model HMGB1 has been shown to activate the neutrophil NADPH oxidase via TLR4 signaling through both MyD88-IRAK4-p38 MAPK and MyD88-IRAK4-Akt pathways with subsequent ROS production [125]. HMGB1 is also a potent neutrophil chemotactic agent in its reduced form when in a heterocomplex with CXCL12 via CXCR4 signaling [126–128]. Hemorrhagic shock in mice causes HMGB1 levels to increase in plasma and lung tissue, with increased pulmonary sequestration of PMN and increased lung permeability observed [129]. Treatment with anti-HMGB1 antibodies prior to hemorrhage prevents neutrophil accumulation in the lungs and reduces lung permeability, demonstrating a role for HMGB1 in neutrophil-mediated acute lung injury following hemorrhagic shock. Similarly, the effects of HMGB1-mediated gut barrier dysfunction in another in-vivo murine HS/R model could be abrogated with anti-HMGB1 antibody and showed a survival benefit, while a murine femur fracture model further demonstrated a critical role for HMGB1/TLR4 signaling in systemic inflammation and remote end-organ (liver) injury in trauma, presumably through neutrophil-mediated effector mechanisms [130, 131].

While primed and activated by DAMPs and inflammatory cytokines early following traumatic injury, the ability of neutrophils to continue responding appropriately to an insult by transmigrating to sites of injury or infection can be overwhelmed in states of ongoing systemic critical illness and injury with incipient immunosuppression. Underlying mechanisms of innate immunodysfunction include reduced adhesion molecule expression secondary to upregulation of iNOS, HO-1, and PPARγ, and desensitization to future priming and activation in a mechanism that depends on GPCR phosphorylation, CXCR2 downregulation and desensitization by FPR1 and TLR2/TLR4 agonism, ongoing CXC stimulation (e.g. IL-8, GRO-α), and BLT1 (LTB_4 receptor) suppression amongst others [33, 50,

55, 132]. Neutrophils become "paralyzed" in such states, rendering them unable to adhere or efficiently chemotax toward sites of infection and injury with increased membrane rigidity and capillary sequestration [33, 49, 132]. In tandem, it has long been recognized that patients with severe trauma and sepsis develop suppressed adaptive immune responses including decreased antigen presentation, macrophage paralysis, diminished T-cell proliferation responses, increased lymphocyte and dendritic cell apoptosis, and a shift from TH1 to TH2 lymphocyte predominance [133]. While the original literature designated this response as a late "compensatory anti-inflammatory response syndrome," or CARS, this paradigm has been challenged recently with increasing evidence to suggest that the adaptive immunosuppressive response occurs both early and simultaneously with increased innate immune inflammatory responses, a notion rooted in decades-earlier observations that critically ill ICU/trauma patients often demonstrate anergy that is strongly associated with sepsis-related mortality [134, 135]. Genome-wide expression analysis of severe blunt trauma patient leukocytes has demonstrated early simultaneous increased expression of systemic inflammatory response, innate immune response, and compensatory anti-inflammatory response genes with concomitant suppression of adaptive immune genes [134]. In lieu of these and other similar observations, a new clinical entity termed "persistent inflammation, immunosuppression, and catabolism syndrome" (PICS) has been introduced that, in part, has resulted from improved surgical and critical care techniques with increased survival of patients early after trauma, sepsis, and organ failure [133]. Subsequent studies have seemingly validated this entity, with genomic analysis showing persistently elevated inflammatory gene expression and decreased adaptive immune gene expression in patients with complicated clinical trajectories following severe blunt trauma [136]. This gene expression data correlated with clinical evidence of persistent inflammation, immunosuppression, and protein depletion, supporting PICS as a predominant clinical phenotype in prolonged critical illness following trauma [136].

In summary, human DAMPs such as MTD' and HMGB1 offer numerous explanations for the "septic" phenotype observed in non-infected trauma victims, with many of their downstream effects being executed by neutrophils and the innate immune system through recognition of these host "danger" signals. At the same time, adaptive immunity is suppressed and patients are at high risk for infectious complications and poor long-term outcomes. DAMPs and their downstream targets, especially neutrophils, are likely an excellent therapeutic target for novel pharmaceutical interventions in trauma and trauma-induced coagulopathy.

Summary Conclusions

In keeping with a focus on traumatic coagulopathy, one can envision from this brief review of neutrophil biology and its cross-talk with the coagulation system how a loss of regulation and negative feedback in neutrophil-mediated responses may play a critical role in propagating inflammation and coagulopathy following traumatic injury. Through DAMP release with subsequent priming, neutrophil activation with the release of toxic ROS and proteases, and NETosis, the neutrophil provides an array of mechanisms by which the innate immune inflammatory response and coagulation system can communicate and lead to unintended endothelial, tissue, and end-organ injury. With further endothelial and tissue injury come additional demands on the coagulation system and further priming and activation of neutrophils. Without proper regulation and return to homeostasis, this positive feedback loop (Fig. 13.1) drives worsening coagulopathy and diffuse inflammatory injury that can have deadly consequences in trauma-induced coagulopathy.

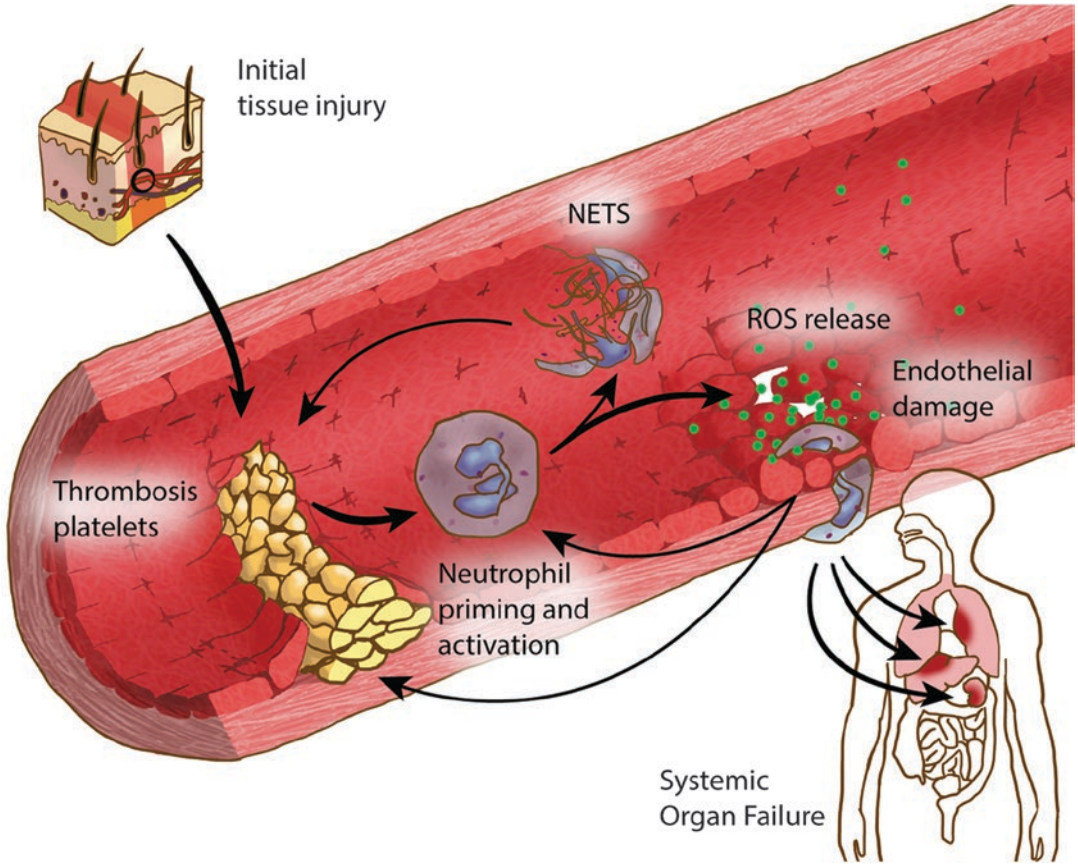


Fig. 13.1 Positive feedback loop between tissue injury, neutrophil activation, thrombosis, and endothelial damage in traumatic coagulopathy. (See text for details. Illustration by Iris Fung, MIT Class of 2017)

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14 Disseminated Intravascular Coagulation

Satoshi Gando and Takeshi Wada

Abbreviations

APTT	Activated partial thromboplastin time
DAMPs	Damage-associated molecular patterns
DIC	Disseminated intravascular coagulation
ED	Emergency department
EPCR	Endothelial protein C receptor
FDP	Fibrin/fibrinogen degradation products
FES	Fat embolism syndrome
FFP	Fresh frozen plasma
GPVI	Glycoprotein VI
HMGB1	High mobility group box 1
ICMA-1	Intercellular adhesion molecule-1
IL	Interleukin
ISS	Injury Severity Score
ISTH	International Society on Thrombosis and Haemostasis
iTBI	Isolated traumatic brain injury
JAAM	Japanese Association for Acute Medicine
MODS	Multiple organ dysfunction syndrome
NETs	Neutrophil extracellular traps
PAI-1	Plasminogen activator inhibitor-1
PAMPs	Pathogen-associated molecular patterns
PRBC	Packed red blood cells
PT	Prothrombin time
SIRS	Systemic inflammatory response syndrome
SIRS	Systemic inflammatory response syndrome
SSC	Scientific and Standardization Committee
TAFI	Thrombin-activatable fibrinolysis inhibitor
TAT	Thrombin and antithrombin complex
TFPI	Tissue factor pathway inhibitor
TIC	Trauma-induced coagulopathy
TNF-α	Tumor necrosis factor α
t-PA	Tissue-type plasminogen activator
VCAM-1	Vascular cell adhesion molecule-1
VWF	von Willebrand factor

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Historical Perspective

Disseminated intravascular coagulation (DIC) was first described clinically in a case of sepsis following trauma and was termed as "DIC syndromes" in 1961 [1]. On the basis of autopsy evidence, MacKay lists trauma as one of the most common causes of DIC and he discusses the pathogenesis of DIC based on a thorough understanding of the clinical courses of coagulation changes seen after trauma [2]. As early as the 1970s, Flute [3] pointed out that trauma stimulates blood coagulation and fibrinolysis concurrently and that fibrinolysis is the compensatory mechanism for fibrin formation in the blood. When this compensating mechanism fails, DIC causes microvascular thrombosis with bleeding and tissue necrosis resulting from a defibrination syndrome. The pathogenesis of DIC in trauma is considered to be due to the entry of thromboplastic materials, such as the products of tissue damage, into the circulation, which in turn lead to platelet aggregation, coagulation activation, and fibrin deposition. If sufficiently severe to induce the consumption of hemostatic components, systemic intravascular coagulation results [2–4]. So, even by the early 1970s, the definition, pathogenesis, clinical aspects, and treatment of DIC as well as the clinical conditions associated with it, such as trauma, had already been described.

Until the early 1980s, whole blood was transfused, which provided adequate amounts of most coagulation factors. As such, the dilution of coagulation factors occurs long after thrombocytopenia develops [5]. Trauma specialists are well aware of the fact that injury and hemorrhagic shock, not hemodilution, induce the early coagulopathy of trauma [6–10]. In 1985, Ordog et al. clearly demonstrated that coagulation abnormalities during hemorrhagic shock are attributable to the trauma itself, independent of fluid and blood replacement; this was reported to most likely be DIC [2, 6, 11]. In the late 1980s, the use of whole blood was almost completely replaced by packed red blood cells (PRBC), which have no coagulation factors. Around a decade later, Hiippala [12] revealed that hypofibrinogenemia develops first and is followed by other coagulation factor deficits and later by thrombocytopenia. Therefore, the use of plasma transfusion became the primary intervention for abnormal bleeding [12]. Despite these changes in transfusion strategies and the importance of Hiippala's report, the incorrect notion that plasma should be transfused late in the resuscitation process continued to be advocated until the early 2000s [13]. This partly explains the prevailing notion of dilutional coagulopathy and the lack of acceptance of an endogenously induced DIC according to trauma or shock itself [14]. Based on this historical perspective, although multiple factors contribute to trauma-induced coagulopathy, it is DIC that is important pathogenetically, with dilution, hypothermia, and acidosis subsequently modifying the processes of DIC.

In 2001, the Scientific and Standardization Committee (SSC) on DIC of the International Society on Thrombosis and Haemostasis (ISTH) announced a definition of DIC based on its clinical and pathological features, in which trauma was acknowledged as an important underlying conditions that evokes DIC [15]. Elucidation of the crosstalk between inflammation and coagulation progressed substantially during the 2000s [16]. Based on the understanding of bidirectional interaction between innate immune inflammation and coagulation, the pathophysiology of DIC in trauma and traumatic shock has been extensively reviewed, resulting in the confident establishment of the role of DIC in trauma [14].

Definition and Diagnosis of DIC

The SSC on DIC of the ISTH defines DIC as an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which, if sufficiently severe, can produce organ dysfunction [15]. The most important aspects of this definition are the "activation of coagulation with loss of localization" and "damage to the microvasculature," referring in turn to systemic thrombin generation in the circulation and to extensive damage to the microvascular endothelial cells that results in insufficient coagulation control. Figure 14.1 shows the basic concept of DIC in which circulating blood is hypercoagulable due to systemic thrombin generation because of insufficient anticoagulation mechanisms, but is hypocoagulable outside the

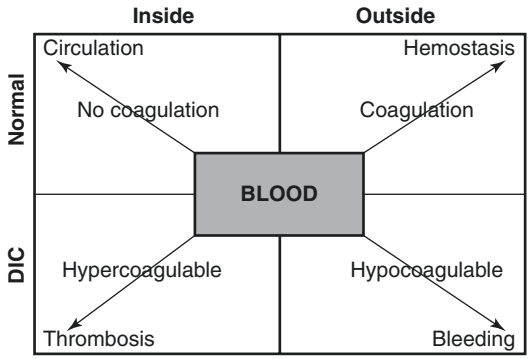


Fig. 14.1 The properties of blood inside and outside the vessels under normal conditions and DIC. In DIC, circulating blood (inside of the vessel) is hypercoagulable due to systemic thrombin generation in response to insufficient anticoagulation mechanisms, but is hypocoagulable outside the vessels, namely, at the site of injury, and clotting is difficult due to consumption coagulopathy [17]. Be aware that situation is completely opposite between normal conditions and pathological DIC. DIC disseminated intravascular coagulation. (Reprinted with modifications, with permission from Clinics in DIC by Matsuda T; Shinkoh-Igaku Shuppan Co., Ltd., Tokyo, 1983)

vessels, namely, at the site of injury, and clotting is difficult due to consumption coagulopathy [17].

The DIC diagnostic criteria of ISTH and the Japanese Association for Acute Medicine (JAAM) have been prospectively validated in critically ill patients, including those with trauma [18–20]. The JAAM DIC scoring system has proven diagnostic validity for DIC in the early phase of trauma and has better diagnostic sensitivity than the ISTH scoring system. In addition, the JAAM DIC score on admission to the emergency department (ED) can independently predict death and the need for massive transfusion in trauma patients [20–22]. These features of the JAAM diagnostic criteria may be dependent on the deletion of fibrinogen, inclusion of sensitive systemic inflammation criteria, and the addition of a dynamic component such as decreasing rate of platelet count. The ISTH scoring system includes a table of the "clinical conditions that may be associated with DIC" as a mandatory clause and restricts the use of the scoring algorithm in patients without underlying diseases. JAAM presents the same table while also adding another table with the title of "clinical conditions that should be carefully ruled out" in order to increase the specificity of the scoring system. Tables 14.1a

Table 14.1a The scoring system for overt disseminated intravascular coagulation (DIC) proposed by the International Society on Thrombosis and Haemostasis (ISTH)

Clinical conditions that may be associated with overt DIC	
Sepsis/severe infection (any microorganism)	
Trauma (e.g., polytrauma, neurotrauma, fat embolism)	
Organ dysfunction (e.g., severe pancreatitis)	
Malignancy	
Solid tumors	
Myeloproliferative/lymphoproliferative malignancies	
Obstetric calamities	
Amniotic fluid embolism	
Abruptio placentae	
Vascular abnormalities	
Kasabach–Merritt syndrome	
Large vascular aneurysms	
Severe hepatic failure	
Severe toxic or immunologic reactions	
Snakebite	
Recreational drugs	
Transfusion reactions	
Transplant rejection	
1. Risk assessment: Does the patient have an underlying disorder known to be associated with overt DIC?	
If yes, proceed; if no, do not use this algorithm	
2. Order global coagulation tests (platelet count, prothrombin time, soluble fibrin monomers, or fibrin degradation products)	
3. Score global coagulation test results	
	Score
Platelet counts (10^9/L)	
<50	2
≥50 < 100	1
≥100	0
Elevated fibrin-related marker (e.g., soluble fibrin monomers/fibrin degradation products)	
Strong increase	3
Moderate increase	2
No increase	0
Prolonged prothrombin time (s)	
≥6	2
≥3 < 6	1
<3	0
Fibrinogen level (g/mL)	
<100	1
≥100	0
4. Calculate score	
5. If >5: compatible with overt DIC; repeat scoring daily	
If <5: suggestive (not affirmative) for non-overt DIC; repeat next 1–2 days	

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Table 14.1b The scoring system for disseminated intravascular coagulation (DIC) by the Japanese Association for Acute Medicine (JAAM)

1. Clinical conditions that may be associated with DIC
(1) Sepsis/severe infection (any microorganism)
(2) Trauma/burn/surgery
(3) Vascular abnormalities
Large vascular aneurysms
Giant hemangioma
Vasculitis
(4) Severe toxic or immunological reactions
Snakebite
Recreational drugs
Transfusion reactions
Transplant rejection
(5) Malignancy (except bone marrow suppression)
(6) Obstetric calamities
(7) Conditions that may be associated with SIRS
Organ destruction (e.g., severe pancreatitis)
Severe hepatic failure
Ischemia/hypoxia/shock
Heat stroke/malignant syndrome
Fat embolism
Rhabdomyolysis
Other
(8) Other
2. Clinical conditions that should be carefully ruled out
A. Thrombocytopenia
(1) Dilution and abnormal distribution
Massive blood loss and transfusion, massive infusion
(2) Increased platelet destruction
ITP, TTP/HUS, HIT, drugs, viral infection, alloimmune destruction, APS, HELLP, extracorporeal circulation
(3) Decreased platelet production
Viral infection, drugs, radiation, nutritional deficiency (vitamin B12, folic acid), disorders of hematopoiesis, liver disease, HPS
(4) Spurious decrease
EDTA-dependent agglutinins, insufficient anticoagulation of blood samples
(5) Other
Hypothermia, artificial devices in the vessel
B. Prolonged prothrombin time
Anticoagulation therapy, anticoagulant in blood samples, vitamin K deficiency, liver cirrhosis, massive blood loss and transfusion
C. Elevated FDP
Thrombosis, hemostasis and wound healing, hematoma, pleural effusion, ascites, anticoagulant in blood samples, antifibrinolytic therapy
D. Other
3. The diagnostic algorithm for SIRS
(1) Temperature > 38 °C or < 36 °C
(2) Heart rate > 90 beats/min
(3) Respiratory rate > 20 breaths/min or $PaCO_2$ < 32 Torr (<4.3 kPa)
(4) White blood cell >12,000 cells/mm^3, <4000 cells/mm^3, or 10% immature (band) forms
4. The diagnostic algorithm

	Score
SIRS criteria	
≥3	1
0–2	0
Platelet counts (10^9/L)	
<80 or more than 50% decrease within 24 h	3
≥80 < 120 or more than 30% decrease within 24 h	1
≥120	0
Prothrombin time (value of patient/normal value)	
≥1.2	1
<1.2	0
Fibrin/fibrinogen degradation products (mg/L)	
≥25	3
≥10 < 25	1
<10	0
Diagnosis	
Four points or more	DIC

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SIRS systemic inflammatory response syndrome, *ITP* idiopathic thrombocytopenic purpura, *TTP* thrombotic thrombocytopenic purpura, *HUS* hemolytic uremic syndrome, *HIT* heparin-induced thrombocytopenia, *APS* antiphospholipid syndrome, *HELLP* hemolysis, elevated liver enzymes, and low platelet, *HPS* hemophagocytic syndrome, *EDTA* ethylenediaminetetraacetic acid, *FDP* fibrin/fibrinogen degradation products

and 14.1b show the ISTH and JAAM DIC scoring systems, respectively [15, 19].

Phenotypes and Time Courses of DIC

DIC can be subdivided into the fibrinolytic and thrombotic phenotypes [14, 23, 24]. DIC with the fibrinolytic phenotype is defined as the coexis-

tence of DIC and systemic pathological hyperfibrin(ogen)olysis in the same insult due to tissue-type plasminogen activator (t-PA) release independent of fibrin clot formation [23, 25]. Both primary fibrinogenolysis independent of fibrin clot and secondary fibrinolysis due to DIC are observed in DIC with the fibrinolytic phenotype. In contrast, the plasminogen activator inhibitor-1 (PAI-1)-mediated inhibition of fibrinolysis is considered to be the cause of DIC with the thrombotic phenotype [17, 24, 26]. The activation of coagulation and an insufficient anticoagulation system are always involved in both types of DIC despite changes in fibrinolysis (Fig. 14.2) [17, 25]. DIC in the early phase of trauma shows the fibrinolytic phenotype, which contributes to oozing-type bleeding and is associated with a poor prognosis [14, 19]. DIC in the late phase of trauma has a thrombotic phenotype that also affects prognosis as it leads to organ dysfunction [17, 24 26].

Use of the DIC diagnostic criteria clearly distinguishes the pathological reaction of DIC from physiological hemostasis and wound healing [14, 17, 27]. Figure 14.3 (left) shows normal changes in hemostasis and wound healing, while Fig. 14.3 (right) shows the abnormal hemostatic responses associated with DIC from immediately after trauma to the late phase of trauma [14, 17, 27].

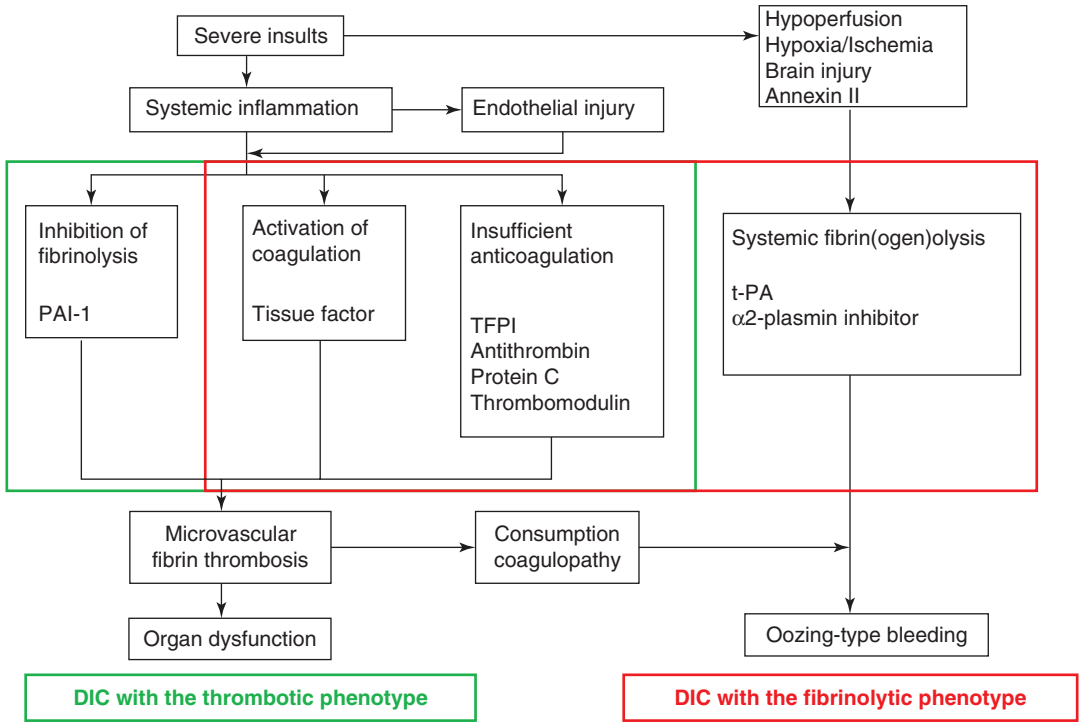


Fig. 14.2 The two phenotypes of DIC. Although the activation of the tissue-factor-dependent pathway as the initial step of the coagulation cascade and the presence of insufficient anticoagulation systems are the same, DIC can be subdivided into the thrombotic (*green line*) and fibrinolytic (*red line*) phenotypes. In DIC with the fibrinolytic phenotype, DIC and systemic pathological hyprefibrin(ogen)olysis coexist. Shock-induced hypoperfusion and global hypoxia/ischemia cause the release of t-PA from endothelial Weibel–Palade bodies. Traumatic brain injury releases t-PA from neurons and other cell types within the central nervous system storing t-PA. Annexin II expression on the promyelocytes accelerates the t-PA activity on plasminogen. All of these conditions produce massive amount of plasmin and consume α2-plasmin inhibitor, leading to hyprefibrin(ogen)olysis. The activation of coagulation and insufficient anticoagulation systems are always involved in both types of DIC. DIC disseminated intravascular coagulation, PAI-1 plasminogen activator inhibitor-1, TFPI tissue factor pathway inhibitor, t-PA tissue-type plasminogen activator. (Reprinted with modifications from reference [25] (Creative Commons Attribution International License))

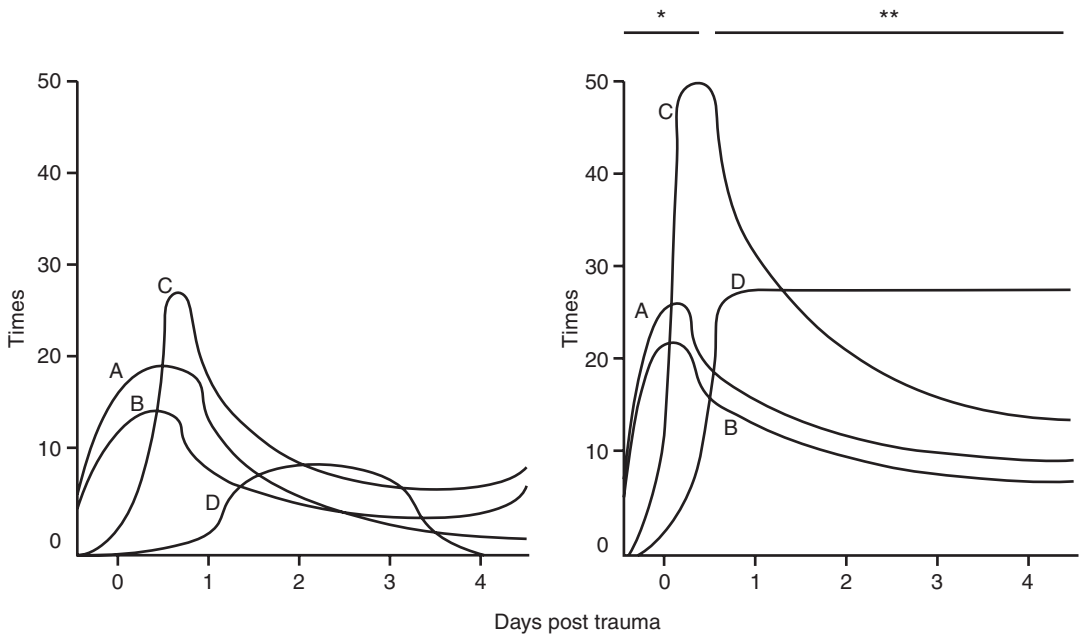


Fig. 14.3 Schematic diagrams of the variations in thrombin activity (*A*, measured by fibrinopeptide A-FPA), plasmin activity (*B*, fibrinopeptide B β15-42-FPBβ15-42), fibrin formation, and secondary fibrinolysis (*C*, D-dimer) from day 0 (in the emergency department) to day 4. *Left*, normal changes in hemostasis and wound healing. There are three phases of fibrinolysis: early activation, impairment (*D*, PAI-1: fibrinolysis shutdown), and reactivation. Normally, both thrombin activity and PAI-1 are completely shut off by days 3–5 after trauma, followed by the reactivation of fibrinolysis. *Right*, pathological changes in DIC. There is time delay between immediate t-PA-induced massive plasmin generation and the induction of PAI-1 mRNA, which causes systemic hyperfibrin(ogen) olysis (* DIC with the fibrinolytic phenotype), followed by impairment of fibrinolysis due to persistent elevation of PAI-1 released from endothelial cells with transcription (** DIC with the thrombotic phenotype). Persistent and systemic thrombin generation always underlies these changes in fibrinolysis. DIC disseminated intravascular coagulation, PAI-1 plasminogen activator inhibitor-1. (Reprinted with modification with permission from [27])

DIC with the fibrinolytic phenotype progresses to DIC with the thrombotic phenotype after a couple of hours.

Pathogenesis of DIC

Bidirectional Interplay Between Innate Immune Inflammation and Coagulation

The bidirectional interplay between innate immune inflammation and coagulation is well recognized [16, 28] and considered the main pathophysiology of DIC [15]. Neutrophil-formed neutrophil extracellular traps (NETs) and cell damage-released damage-associated molecular patterns (DAMPs), especially histones, synergistically play pivotal roles in inflammation, platelet and coagulation activation, insufficient anticoagulation, and inhibition of fibrinolysis, all of which act as main pathogenesis of DIC [29]. Trauma can induce systemic inflammatory response syndrome (SIRS), which is characterized by proinflammatory cytokine release and the activation of neutrophils and the endothelium, processes that are recognized as innate immune inflammation and give rise to the activation of coagulation associated with microvascular thrombosis, namely, DIC [14].

Hemostasis at the Site of Injury

Innate immune cells have evolved cell-specific prothrombotic pathways that are activated after

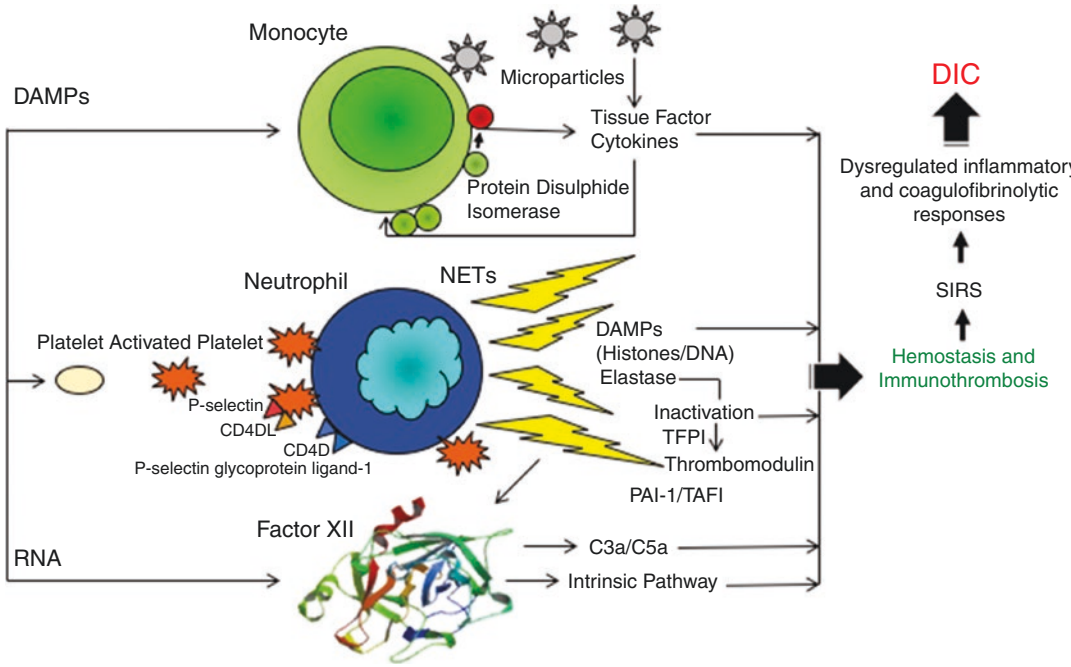


Fig. 14.4 The pathophysiological processes of local hemostasis, immunothrombosis, and systemic DIC. The aims of fibrin thrombosis at the local site of injury are both to stop bleeding and to impede the dissemination of DAMPs, protecting the host from DAMPs. DIC results when local hemostasis and immunothrombosis are no longer able to anchor thrombin or to restrict the spread of DAMPs at the injured site. Detailed descriptions are given in the text. DAMPS damage-associated molecular patterns, DIC disseminated intravascular coagulation, NETs neutrophil extracellular traps, PAI-1 plasminogen activator inhibitor-1, SIRS systemic inflammatory response syndrome, TFPI tissue factor pathway inhibitor. (Reprinted from reference [25] (Creative Commons Attribution International License))

insults and operate in intact blood vessels to protect the host from nonself (pathogen-associated molecular patterns, PAMPs) and altered-self (DAMPs); this concept is referred to as immunothrombosis [30]. During the responses to PAMPs and DAMPs, monocytes and their microparticles express tissue factor, which activates the extrinsic coagulation pathway [31, 32]. Neutrophils are recruited to the sites of inflammation and are activated [33], and then release NETs, which are comprised of a matrix of DNA, histones, nucleosomes, and neutrophil elastase, thereby promoting thrombosis [34]. Histones induce platelet activation and also promote thrombin generation both by the recruitment of platelet and the impairment of thrombomodulin-dependent protein C activation [35–37]. NETs and DNA can also activate the intrinsic coagulation pathway by activating FXII to form FXIIa [38], which then promotes the activation of complement pathways. The generated C3a and C5a further promote thrombosis and platelet activation [39]. In addition, extracellular RNA derived from damaged cells constitutes a procoagulant cofactor for the activation of the FXII/FXI-induced coagulation pathway [40]. The neutrophil elastase that is present on NETs induces the degradation and inactivation of tissue factor pathway inhibitor (TFPI) [41, 42] and the thrombomodulin expressed on the endothelium [43, 44]. These processes further promote thrombin generation. The normal hemostatic processes begin after the exposure of subendothelial tissue factor into the bloodstream. In addition to these processes, immunothrombosis may also play a role in hemostasis at the site of injury.

Immunothrombosis and hemostatic thrombosis at injury sites impede the dissemination and tissue invasion of thrombin, DAMPs, and damaged cells themselves [30, 42]. In trauma, throm-

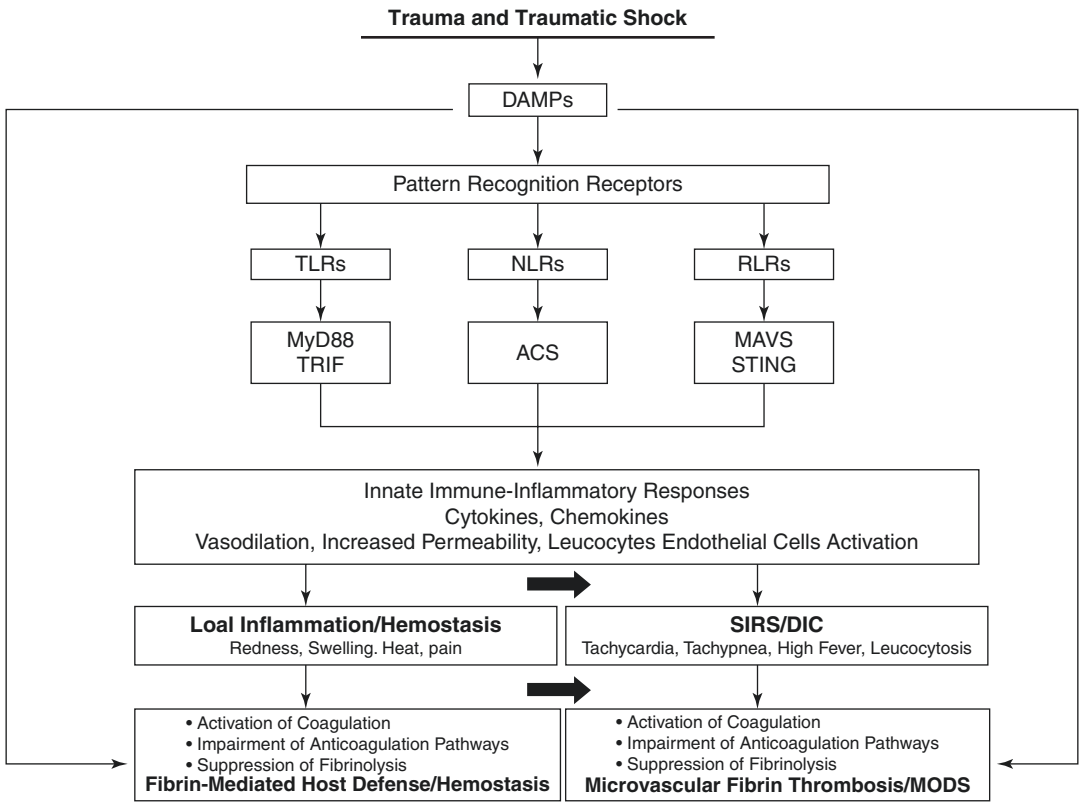


Fig. 14.5 Trauma elicits nonspecific innate immune inflammatory responses that limit and repair tissue damage after insults. The figure depicts a simplified schematic representation of the engagement of pattern recognition receptors by DAMPs and their signaling through the adaptor proteins. This cascade promotes the transcription of several pro-inflammatory cytokines and chemokines, leading to local and systemic inflammatory responses. Local inflammation begins as an adaptive host response, serving to promote host defense and physiologic hemostasis and wound healing. Spillover of the inflammatory cytokines into the circulation elicits SIRS, which activates systemic coagulation, suppresses fibrinolysis, and overwhelms the anticoagulant control mechanisms that restrict hemostasis locally, giving rise to DIC. Importantly, DAMPs themselves activate coagulation and impair anticoagulation pathways through endothelial damage. ACS apoptosis-associated speck-like protein containing caspase recruit domain, DAMPs damage-associated molecular patterns, DIC disseminated intravascular coagulation, MAVS mitochondrial antiviral signaling, MODS multiple organ dysfunction syndrome, MyD88 myeloid differentiation factor 88, NLRs nucleotide-binding oligomerization domain containing receptors, RLRs retinoic acid inducible gene-I-like receptors, SIRS systemic inflammatory response syndrome, STING stimulator of interferon gene, TRIF toll/IL-1 receptor homology domain-containing adaptor inducing interferon beta, TLRs toll-like receptors. (Reprinted with modification with permission from [46])

bin escaping into the circulation from the injury sites is controlled by antithrombin, TFPI, and thrombomodulin present in intact endothelial cells, as depicted in the cell-based model of hemostasis [45]. However, when systemic inflammation caused by both extensive injury and shock overwhelms these control mechanisms that restrict hemostasis and inflammation locally, DIC ensues [45]. Figures 14.4 and 14.5 outline these processes [46].

Systemic Activation of Coagulation

The major DAMPs released into the extracellular environment following tissue injury are histones, mitochondrial DNA, nucleosomes, and high mobility group box 1 (HMGB1) [47]. Mitochondrial DNA at levels thousand times higher than in healthy volunteers have been observed at a median 93 min after trauma [48]. Elevated levels of mitochondrial DNA have been

shown to be associated with SIRS and organ dysfunctions and correlated with the Injury Severity Score (ISS) in trauma patients [48–50] . Elevated levels of histones and HMGB1 have been seen in severely injured trauma patients within 30 min of trauma or immediately after arrival at the ED [51–53]. Toxic levels of histones insult cells, leading to endothelial injury (elevation of soluble thrombomodulin), platelet aggregation, thrombin generation (increases in thrombin and antithrombin complex (TAT)), interleukin-6 (IL-6) release, and NETs formation [29, 53]. NETs bear the exposed histones, and the histones in turn activate neutrophils to release NETs, thereby propagating mutual interplay and leading to further thrombin generation and DIC [29]. These processes result in edema, microvascular thrombosis, and neutrophil congestion in the lungs. The HMGB1 released by damaged and inflammatory cells at the injury site promotes the development of microvascular thrombosis [54]. An important point is that HMGB1 inhibits the anticoagulant protein C pathway mediated by the thrombin-thrombomodulin complex and stimulates tissue factor expression on monocytes. Histones also reduce the cofactor activity of both soluble and endothelial thrombomodulin and impair protein C activation, thereby stimulating plasma thrombin generation [37].

Tumor necrosis factor α (TNF-α) and IL-6 are elevated immediately after histone infusion [53, 55]. In fact, TNF-α as well as IL-1β is increased on arrival at the ED in trauma patients with complicated DIC [56] and an IL-6 surge occurs within 2 h of trauma [57]. This early release of IL-6 suggests it was most likely released from presynthesized stores [53]. IL-6 is the most important driver of tissue factor expression on monocytes and endothelial cells [58]. TNF-α and IL-1 also have been shown to elicit tissue factor formation and expression on the surface of monocytes and endothelial cells [59]. These inflammatory cytokines subsequently block the protein C anticoagulant pathway by downregulating thrombomodulin and the endothelial protein C receptor (EPCR) on the endothelium [59]. Furthermore, these inflammatory cytokines activate neutrophils and endothelial cells, and the activated neutrophils release neutrophil elastase, which can cleave thrombomodulin, leading to the release of soluble thrombomodulin from the endothelium in a less active form [43, 44, 59, 60].

Although the mechanisms involved in microparticle formation *in vivo* remain essentially unknown, blood contains microparticles derived from a variety of cell types, including platelets, monocytes, and endothelial cells [61]. All microparticles are procoagulant because they provide a membrane surface for assembly of coagulation protease cascades [62]. In severe trauma, activated platelets enhance the microparticle formation associated with platelet and leucocyte interaction [63]. Increased microparticle formation and thrombin generation are observed immediately after trauma and correlate with ISS [64].

These lines of evidence clearly indicate that activated neutrophil-released NETs, DAMPs from injured cells and tissues, the DAMP-induced release of inflammatory cytokines, and microparticle formation synergistically hamper the anticoagulation pathways and activate coagulation, leading to SIRS and systemic thrombin generation, namely, DIC, immediately after trauma [29, 65].

Impairment of Anticoagulation Pathways and Endothelial Injury

TFPI

The highly activated tissue factor-dependent pathway is not sufficiently prevented by normal TFPI levels in DIC patients after trauma because neutrophil elastase cleaves TFPI within the polypeptide that links the first and second Kunitz domains [66]. This impairs the ability of TFPI to neutralize both FXa and the tissue factor/FVIIa complex. This finding suggests that tissue factor and tissue factor/FVIIa complex are continuously formed at a rate that normal TFPI inhibition cannot match in DIC patients after trauma [66, 67].

Thrombomodulin, Glycocalyx, and Endothelium

Increased levels of neutrophil elastase, a marker of neutrophil activation, and soluble thrombomodulin have been confirmed in patients with DIC and those with severe traumatic injuries [53, 68, 69]. Increased levels of soluble intercellular adhesion molecule-1 (ICMA-1) and soluble vascular cell adhesion molecule-1 (VCAM-1), markers of endothelial activation, in DIC patients compared with non-DIC patients after trauma have been also observed [70]. These results indicate that neutrophil and endothelial activation followed by endothelial injury [69]. Soluble thrombomodulin can be formed by the limited proteolysis of endothelial cell membrane thrombomodulin by neutrophil elastase without any evidence of active secretion [43, 44]. The amount of soluble thrombomodulin correlates with the degree of endothelial injury [45]. Moreover, early elevation of TNF-α and IL-1β in DIC patients after trauma causes thrombomodulin downregulation in the endothelium [44, 45, 56]. Traumatic shock-induced endothelial hypoxia leads to a reduction in thrombomodulin and the suppression of thrombomodulin mRNA in the endothelium [71, 72]. Therefore, the high soluble thrombomodulin levels in DIC patients suggest a loss of functional endothelial thrombomodulin due to endothelial injury. In addition, soluble thrombomodulin has only 20% of the activity of normal endothelial thrombomodulin [73]. Taken together, these results suggest that endothelial injury and functional loss of both soluble and endothelial thrombomodulin occurs in DIC after trauma.

Capillary leak syndrome and an insufficient anticoagulation system are the main characteristics of DIC [15]. Glycocalyx covering the endothelial cell surface helps maintain the normal vascular physiology, including vascular permeability and coagulation [74]. Glycocalyx consists of cell adhesion molecules (glycoproteins) and proteoglycans such as syndecan and glypican, which bind to the endothelial cells, with proteoglycans connecting to glycosaminoglycan. Glycocalyx perturbation and shedding increases vascular permeability and activates coagulation due to insufficient anticoagulation mechanisms, such as antithrombin, TFPI, and endothelial thrombomodulin [74, 75]. Trauma with SIRS is a major cause of glycocalyx perturbation [74, 76]. In patients with trauma, glycocalyx shedding and endothelial injury evaluated with soluble forms of syndecan-1 and thrombomodulin occur within minutes after injury [77]. These results suggest that disrupted glycocalyx and endothelium lead to increased vascular permeability and insufficient anticoagulation mechanisms in trauma patients [75, 78].

Protein S and Protein C

Low levels of protein C activity have been repeatedly confirmed from the early to late phases of DIC after trauma [17, 19, 21, 68]. The thrombin-thrombomodulin complex activates protein C to generate activated protein C. For activated protein C to function, it must form a complex with both protein S and EPCR. The anticoagulant activity of protein S is neutralized by the formation of a complex with complement C4b binding protein (C4bBP). Increased levels of C4bBP as a consequence of the acute phase reaction following inflammatory insults cause a relative protein S deficiency, which contributes to a procoagulant state and lethal DIC [79]. Lower levels of protein S activity associated with thrombin generation (prothrombin fragment 1+2, PF1+2) have been demonstrated in trauma patients immediately after arrival at the ED [9].

Activated protein C is immediately inactivated by protease inhibitors, such as the protein C inhibitors, α1-antitrypsin, α2-antiplasmin, and α2-macroglobulin. In cases of DIC due to trauma, lower protein C and protein S levels, relative protein S deficiency, impaired functions of both soluble, and endothelial thrombomodulin are all implicated in the insufficient conversion of protein C to activated protein C and the inability of activated protein C to function normally. Increases in activated protein C levels do not indicate a shutoff of thrombin generation. In fact, the elevated activated protein C levels (~10 ng/mL) did not reach a concentration sufficient to inhibit thrombin generation (70–80 ng/mL) in severely injured trauma patients with tissue

hypoperfusion [80, 81]. Neither a systematic review of clinical studies nor a review of an experimental trauma model showed a cause and effect relationship between activated protein C and the suppression of coagulation or increased fibrinolysis [82, 83].

Antithrombin

Antithrombin inactivates thrombin and inhibits several proteases in both the extrinsic and intrinsic coagulation pathways, including FIXa, FXa, FXIa, and FXIIa. Thus, a reduction in antithrombin can markedly influence the coagulation processes and is a potential risk factor for thrombosis [84]. Insufficient levels of antithrombin, compared with the potential for thrombin generation in the prothrombin complex concentrate, induced DIC in a pig model of coagulopathy with blunt liver injury [85]. The severity of injury and tissue hypoperfusion are major contributors to the reduction in antithrombin in trauma [86, 87]. Low antithrombin levels are a significant predictor of thromboembolic complications, including deep vein thrombosis and DIC [88]. Extremely low levels have been observed in cases of trauma with DIC immediately after arrival at the ED [8, 22] and for several days thereafter [8, 22, 89].

Two studies showed that a decreased ability to localize hemostasis at the wound site and subsequently generate thrombin systemically results from decreased antithrombin levels in patients with DIC and coagulopathy immediately after trauma [90, 91]. Similarly, a multiple regression analysis demonstrated that antithrombin levels are an independent determinant of high soluble fibrin levels, a marker of thrombin generation and activity, in DIC patients after trauma [89].

These findings clearly indicate that the availability of the TFPI, antithrombin/glycosaminoglycan system, and thrombomodulin/protein C system is far too low for the regulation of thrombin generation in DIC patients. Furthermore, elevated soluble syndecan-1 and soluble thrombomodulin levels suggest the presence of extensive damage to the microvasculature glycocalyx and endothelium. All of these changes give rise to systemic thrombin generation, leading to DIC. Figure 14.6 summarizes these changes.

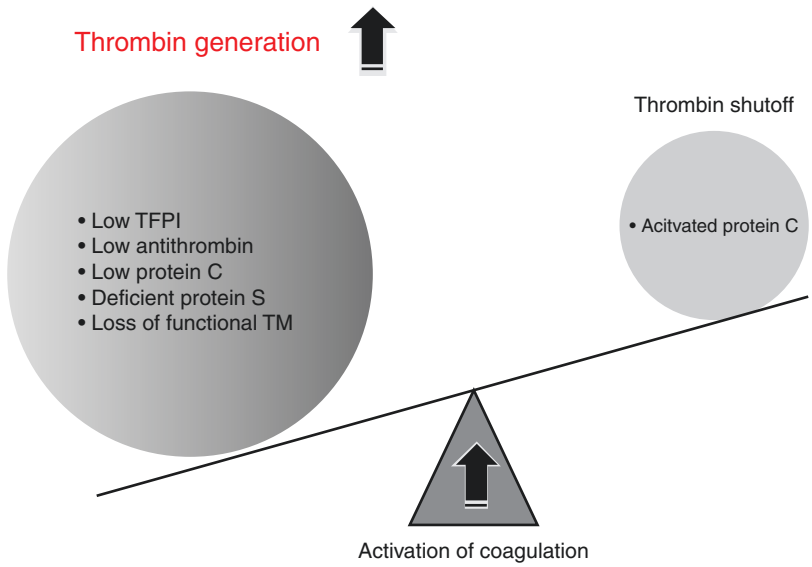


Fig. 14.6 The balance between thrombin generation and inhibition. DIC occurs when there is an imbalance between thrombin generation and inhibition. Insufficient coagulation control mechanisms contribute to massive thrombin generation in the circulation, which overwhelms activated protein C-mediated inhibition of thrombin generation. Namely, systemic thrombin generation associated with insufficient anticoagulation mechanisms gives rise to DIC. DIC disseminated intravascular coagulation, TFPI tissue factor pathway inhibitor, TM thrombomodulin. (Reprinted from reference [25] (Creative Commons Attribution International License))

Thrombin Generation in the Systemic Circulation

Soluble fibrin and fibrinopeptide A are regarded as accurate markers of thrombin generation and activity because both are formed as a result of the direct action of thrombin on fibrinogen, which is followed by fibrin formation. Extremely elevated levels of fibrinopeptide A have been noted in trauma patients with DIC immediately after arrival at the ED [8]. In addition, higher levels of these molecular markers of thrombin generation in the early phase of trauma have been repeatedly confirmed [9, 14, 68]. In two studies, Dunbar and Chandler observed excessive non-wound-related thrombin generation in trauma patients with both DIC and acute coagulopathy of trauma immediately after arrival at the hospital [90, 91]. Their first study showed marked systemic thrombin generation due to circulating procoagulants that initiate thrombin generation systemically, as well as reduced ability to localize hemostasis at the wound site due to the loss of antithrombin. Their second study found that tissue factor activity accounted for approximately 80% of all procoagulant activity. Reports showing a significant correlation between tissue factor and the markers of thrombin generation and microparticle formation by activated platelets support these results [63, 92]. Furthermore, an experimental study completely reproduced the findings from the above two studies showing circulating procoagulants and low levels of antithrombin inducing systemic thrombin generation [93].

The overall function of the thrombomodulin/protein C anticoagulant pathway can be precisely evaluated by measuring prothrombinase activity [94]. Prothrombinase is a complex comprising FXa, FVa, phospholipids, and Ca^{2+} and it is the major determinant of thrombin generation from prothrombin. Prothrombinase activity, measured as the thrombin generation rate, decreases in proportion to the amount of thrombin-thrombomodulin complex-induced formation of activated protein C and the subsequent inactivation of FVa [94, 95]. DIC patients after trauma have shown normal prothrombinase activity associated with higher levels of soluble fibrin [89]. These findings suggest that the inhibition of the prothrombinase activity caused by activated protein C-mediated anticoagulation does not overwhelm the activation of the tissue factor-induced systemic thrombin generation or its activation in trauma patients with DIC. This imbalance between thrombin generation (soluble fibrin) and its inhibition (prothrombinase activity) is due to insufficiency in the other anticoagulant mechanisms, such as TFPI and antithrombin, and impaired thrombomodulin function due to endothelial injury [89].

Consumption Coagulopathy

The consumptive processes in DIC reflect the multiple actions of thrombin. Increased thrombin generation accounts for decreases in platelets, fibrinogen, FII, FV, FVIII, and FXIII in acute consumption, and the rapid clearance of activated clotting factors in vivo accounts for decreases in other clotting factors such as FIX and FX [23]. Thrombin induces the release of t-PA from endothelial cells, leading to plasmin generation. If plasmin is formed sufficiently in the circulation, it degrades fibrinogen, FV, and FVIII. These lines of evidence support the rapid consumption of thrombin-sensitive hemostatic factors, including platelets, fibrinogen, and factors V, VIII, and XIII. In pre-DIC and DIC, sensitive and rapid decreases in the levels of FV and FVIII have been observed as a result of thrombin-mediated protein C activation [96, 97]. In DIC due to trauma, platelets are sometimes consumed slowly due to marginalization in blood vessels and release from storage in organs such as the spleen, liver, and lungs [12, 14, 17]. FVIII is known to paradoxically increase in response to clinical insults, including trauma, due to release of von Willebrand factor (VWF) from the endothelial Weibel–Palade bodies [98] and the acute phase behavior of FVIII. VWF immediately interacts with FVIII, serving to prolong the plasma half-life of FVIII [99]. The consumption of coagulation factors prolongs both the prothrombin time (PT) and activated partial thromboplastin time (APTT); however, the APTT is sometimes normal or even

shortened because of the interactions between FVIII with VWF in spite of the prolonged PT in DIC patients.

In cases of trauma with DIC, prolonged PT reflects a decrease in FV, to a lesser extent decreases in factors II, VII, and X and decreases in fibrinogen levels immediately to several days after trauma [8, 14, 17, 19, 20, 56, 68, 89, 92]. Prolonged APTT, which reflects a decrease in factors V and VIII, and fibrinogen, has also been confirmed immediately after trauma in DIC patients [8]. FVII antigen has been demonstrated to be consumed at a relatively slow speed for about 8 h in a rabbit model of DIC [100]. Importantly, the FVIIa levels increased to 120% within 2 h after DIC induction, before declining thereafter. Furthermore, FXIII and α2-plasmin inhibitor levels showed marked decreases in DIC patients on arrival at the ED [101].

Activation and Suppression of Fibrinolysis

Early Phase

DIC and pathological systemic fibrin(ogen)olysis sometimes coexist following the same insult, such as trauma, and are referred to as DIC with the fibrinolytic phenotype [23, 25]. Hypoxia and thrombin are two major strong stimuli to induce the release of t-PA from endothelial Weibel–Palade bodies in the endothelial cells [98]. Traumatic shock-induced tissue hypoperfusion causes t-PA to be released from the endothelial Weibel–Palade bodies due to endothelial hypoxia [98]. Massive thrombin generation immediately after trauma also releases t-PA from the endothelial Weibel–Palade bodies [98]. Traumatic brain injury releases t-PA from neurons and other cell types within the central nervous system storing t-PA [17, 25, 102] (Fig. 14.2). These immediate t-PA releases generate massive plasmin and consume α2-plasmin inhibitor, leading to systemic hyperfibrin(ogen)olysis in addition to DIC-induced secondary fibrinolysis [23, 25, 98]. In the early phase of trauma, there is a few-hours' time difference between the immediate release of t-PA from the endothelium and the later expression of PAI-1 mRNA, leading to an extreme imbalance in these molecules, which strengthens the systemic hyperfibrin(ogen)olysis [103–105]. In support of this imbalance, the levels of PAI-1 antigen and activity were found to be almost identical in patients with and without DIC immediately after trauma, while the levels of t-PA and plasmin and α2-plasmin inhibitor complex, a marker of plasmin generation, were significantly increased only in DIC patients [8, 17, 56, 89]. Figure 14.7 depicts the pathomechanisms of DIC with the fibrinolytic phenotype [17].

In addition to plasmin, neutrophil elastase-mediated fibrinolysis is also involved in the pathogenesis of fibrin(ogen)olysis in DIC with the fibrinolytic phenotype [68]. The lower levels of α2-plasmin inhibitor, FXIII, and fibronectin in DIC patients suggest that there is insufficient inhibition of plasmin, impaired crosslinking of fibrin, and delayed wound healing, leading to fragile fibrin formation associated with persistent bleeding [8, 101]. A study showing tissue factor-induced fibrin(ogen)olysis without tissue hypoperfusion suggests that secondary fibrinolysis caused by a massive amount of fibrin formation-induced t-PA release may also have a role in DIC with the fibrinolytic phenotype [106]. Importantly, thrombomodulin-mediated thrombin-activatable fibrinolysis inhibitor (TAFI) activation may not have an important role in the pathogenesis of fibrin(ogen)olysis immediately after trauma [68], which indirectly supports thrombomodulin/protein C pathway impairment.

Increased fibrinolysis, as well as the activation of coagulation in trauma, has long been recognized [3, 107]. These changes were reconfirmed in a study demonstrating increased thrombin generation, and fibrinogen and antithrombin consumption, as well as increased t-PA levels and plasmin generation along with α2-plasmin inhibitor consumption, all of which coincided with DIC with the fibrinolytic phenotype [108].

Late Phase

Immediate t-PA-induced fibrinolysis is usually followed by PAI-1-induced suppression of fibrinolysis. After completion of hemostasis, the activation of coagulation and PAI-1 disappears

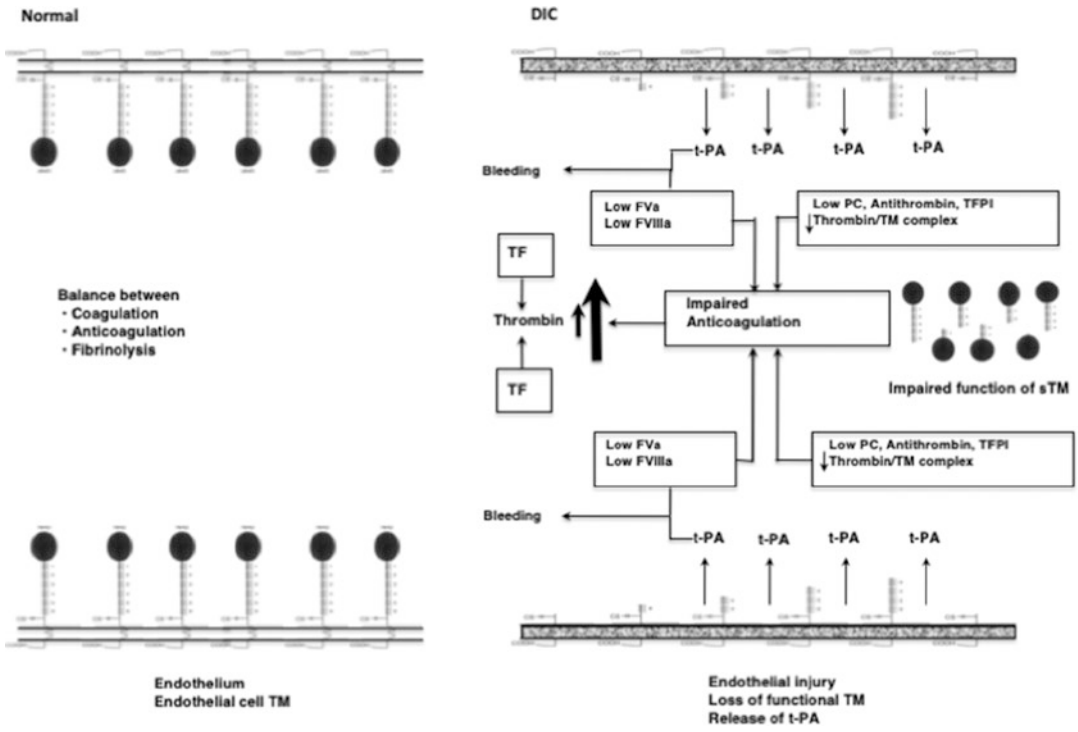


Fig. 14.7 Mechanisms of DIC with the fibrinolytic phenotype. *Left*, there is a balance between activation of coagulation, anticoagulation, and fibrinolysis during physiological hemostasis; *right*, DIC with the fibrinolytic phenotype. Consumption induced decreases in protein C, antithrombin, and TFPI and functional loss of both soluble and endothelial thrombomodulin severely impairs anticoagulation, enhancing systemic thrombin generation. Systemic thrombin generation and shock-induced hypoperfusion stimulate the release of t-PA from endothelial Weibel–Palade bodies. Disseminated fibrin thrombosis-induced endothelial hypoxia also releases t-PA from endothelial cells. TM thrombomodulin, sTM soluble TM, TF tissue factor, PC protein C, TFPI tissue factor pathway inhibitor. (Modified with permission [17])

completely and fibrinolytic reactivation occurs to degrade excess fibrin clots in physiological wound healing [8, 14, 17, 27] (Fig. 14.3 (left). However, persistently high PAI-1 elevation continues until day 5 after injury in DIC patients, which is referred to as DIC with the thrombotic phenotype [8, 14, 17, 27, 56] (Fig. 14.3 (right). When uncontrolled, DIC with the fibrinolytic phenotype in the early phase of trauma continuously progresses to DIC with the thrombotic phenotype in the late phase of trauma [109]. DIC severity and the presence of organ dysfunction on the day of injury are involved in the pathogenesis of this continuous progression. During the thrombotic stage, increased fibrinolysis, observed as elevated D-dimer levels, cannot match the massive fibrin formation, leading to microvascular thrombosis-induced hypoperfusion and impaired oxygen delivery, which in turn give rise to multiple organ dysfunction syndrome (MODS) [25, 104, 110]. Figure 14.3 illustrates these processes [27].

Platelet Activation and Exhaustion

Activated platelet and vessel wall interactions contribute to monocyte-induced tissue factor expression, NETs-mediated activation of coagulation, and impairment of anticoagulant mechanisms, leading to microvascular clot formation in DIC [29, 30, 111]. Activation followed by the reduced function of platelets, which is associated with thrombocytopenia observed in DIC, has been attributed to platelet exhaustion due to

depletion of the storage pool [112, 113]. A systematic review confirmed robust evidence of increased activation of platelets in DIC patients compared with non-DIC patients [114]. Although the platelet counts need to be adjusted, a lower platelet aggregation function in DIC patients than non-DIC patients and healthy controls was also observed in a systematic review. Importantly, these results were consistent across different etiologies of DIC, including trauma [114].

Thrombin and adrenaline have been well-known activators of platelets. Histones directly activate platelets by changing calcium transient followed by aggregation and consumption in both *in vivo* and *in vitro* studies [29, 35, 115]. Histone-associated thrombocytopenia in critically ill patients, including those with trauma, has also been confirmed [116]. High levels of thrombin, adrenalin, and histones have been confirmed in trauma patients, especially those associated with DIC [8, 52, 53, 89, 117]. Trauma patients associated with coagulopathy defined as prolonged PT ≥ 18 seconds showed platelet activation with an impaired function [118, 119]. Platelets showed dysfunction to multiple agonists in trauma patients. In this study, soluble plasma factors, such as soluble fibrin, were suggested to be the cause of dysfunction [118]. The dysfunctional ability to aggregate, while the normal α granule secretory function is preserved, suggests intrinsic platelet or integrin dysfunction in trauma [119].

Thrombin action on fibrinogen produces fibrin monomer, and a part of this monomer immediately binds to fibrinogen to form the complex "soluble fibrin," an established maker of a DIC diagnosis. Extremely high levels of soluble fibrin in both trauma patients and experimental traumatic rat models associated with DIC have been observed [89, 93]. Soluble fibrin downregulates platelet glycoprotein VI (GPVI) and causes the acquisition a platelet GPVI signaling defect, leading to dysfunction of platelets [120]. The results indicate a significant association of thrombin-induced formation of soluble fibrin with platelet activation and dysfunction in trauma associated with DIC.

DIC and Microvascular Thrombosis

Trauma and Hemorrhagic Shock

Histological evidence of microvascular thrombosis in DIC, especially in DIC with the thrombotic phenotype, has been reported by clinical, experimental, and autopsy studies [110]. Evidence of DIC with the fibrinolytic phenotype is rarely available in humans and was extensively debated during the 1960s and 1970s [6]. These debates on the inconsistency of thrombus formation had come about because of the existence of hyperfibrin(ogen)olysis in the early phase of trauma and hemorrhagic shock. However, fibrin thrombosis [121], vein thrombi formation [122], platelet aggregation, and emboli formation [123, 124] were repeatedly confirmed in hemorrhagic shock and trauma. Eeles and Sevitt confirmed the existence of disseminated microvascular thrombosis from immediately to 12–48 h after trauma and emphasized that microvascular thrombosis was quite frequent within 3 h of injury in severely injured patients [125]. Subsequently, platelet and fibrin thrombosis became more evident during antifibrinolytic therapy using tranexamic acid in a dog model of hemorrhagic shock [126]. It should be emphasized that the authors of a report expressing negative opinions about DIC did in fact conclude that some fibrin thrombi were observed in their histological study [127]. Importantly, signs of inflammation, microthrombus, and embolus formation have also been observed within 24 h of injury in human studies [128, 129].

Isolated Traumatic Brain Injury

Publications have demonstrated that the coagulopathy of isolated traumatic brain injury (iTBI) coincides with the definition of DIC proposed by the ISTH, namely, intravascular activation of coagulation, with loss of localization of coagulation, and damage to the microvasculature [15, 130, 131]. Therefore, if coagulofibrinolytic changes after iTBI are sufficiently severe, they give rise to DIC [132].

Microthrombi are frequently present in the brains of iTBI patients who died within 24 h of injury and were found to be associated with marked changes in platelet count, coagulation, and fibrinolysis markers [130]. More importantly, systemic microthrombi were seen in the spinal cord, liver, lungs, kidneys, colon, and pituitary gland, indicating the presence of DIC in 88% of patients after autopsy [130]. Stein et al. examined brain tissue from several sources, including animal models and patients with contused brain tissue removed during surgical decompression, and found a high correlation between the severity of coagulopathy and the presence of intravascular thrombosis, confirming the association between intravascular thrombosis and DIC [131, 133]. These investigators also demonstrated a strong link between intravascular thrombosis and the area of ischemic changes and neuronal death [131, 134]. An animal experiment confirmed that the immediate posttraumatic decrease in peri-contusional blood flow is caused by platelet activation and subsequent microthrombosis in the cerebral circulation [135]. These lines of evidence clearly indicate that a DIC processes occurring immediately after iTBI can contribute to secondary brain injury.

Fat Embolism Syndrome

Fat embolism syndrome (FES) typically occurs 12–36 h after long bone and pelvic fractures, although fulminant cases immediately after injury have also been reported. Several lines of evidence indicate the presence of DIC in patients with FES [136, 137].

Saldeen et al. observed morphological changes in pulmonary, cerebral, and systemic fat emboli that are associated with pathophysiological characteristics of DIC [138]. Furthermore, fibrin thrombosis in pulmonary vessels has been confirmed more often in patients with FES than in other post-traumatic cases, especially in those in whom FES was considered to be the only explanatory cause of death [139]. The presence of fibrin thrombi in lung vessels was also confirmed in patients with FES and acute respiratory distress syndrome [140]. Hyaline microvascular thrombosis and aggregates of platelets, indicating fibrin thrombi and intravascular coagulation, have been reported in both the cerebral arteries and veins in post-traumatic FES patients [141]. Histopathological analysis of FES in a living body revealed intravascular thrombus formation, with the thrombus consisting of fibrin as well as erythrocytes and leukocytes with lipid granules [142].

DIC and MODS

DIC in association with SIRS contributes to microvascular thrombosis and subsequent MODS leading to poor outcome [110, 111]. Inflammatory cytokine-mediated neutrophil-endothelial cell interplay and crosstalk between inflammation and coagulation via protease-activated receptors play important roles in microvascular fibrin thrombosis [110]. A reduction in oxygen delivery due to disseminated thrombosis and tissue dysoxia due to neutrophil activation-induced endothelial injury contributes to the development of MODS [110]. Furthermore, evidence suggests that DAMPs, especially histones, and NETs are major mediators of MODS in patients with DIC [29, 111]. These mediators contribute to MODS through direct injury to cells in different organs and activation of both inflammation and coagulation. Circulating histones are major mediators of MODS in critical illness. NETs formation independently predicts DIC development and has shown significant association with MODS and mortality in critically ill patients [143, 144].

Trauma-released mitochondrial DNA (DAMPs) causes SIRS associated with elevation of inflammatory cytokines and then activates neutrophils, leading to organ injury [48]. Increased histones immediately after trauma

induce IL-6 release, NETs formation, platelet aggregation, systemic thrombin generation, and endothelial injury. These changes induce microvascular thrombosis and capillary leakage leading to DIC followed by MODS [53]. DIC patients have repeatedly shown a higher prevalence of SIRS and MODS and worse outcome than non-DIC patients after trauma [20, 56, 66, 69]. The combined activation of inflammation and coagulation plays an important role in MODS and poor outcome in association with neutrophil activation and endothelial injury in patients with DIC after trauma [70]. DIC-associated SIRS more than 3 three days after trauma is a strong determinant of MODS and a poor prognosis [145]. In this study, the likelihood ratio of DIC within 12 h after trauma predicting MODS was 11.6. Importantly, DIC with the fibrinolytic and the thrombotic phenotypes equally contributes to MODS and a poor prognosis among trauma patients [20, 70].

Animal Models of DIC

Noble–Collip drum shock-induced polytrauma without significant hemorrhage has been used to mimic lethal traumatic injury [146]. The model reproduces typical DIC with the fibrinolytic phenotype, with animals exhibiting a decreased platelet count, prolonged PT and APTT, decreased fibrinogen and antithrombin levels, and elevated fibrin/fibrinogen degradation product (FDP) levels [146–148]. Furthermore, elevated t-PA levels, shortened euglobulin lysis time, and decreased α2-plasmin inhibitor levels indicate immediate activation of the fibrinolytic system [147, 148]. Decreases in the levels of FXII, prekallikrein, and CH50 suggest the activation of both the intrinsic coagulation pathway and the complement system [148]. Immediately after Noble–Collip drum trauma, tissue factor increases in the circulation, and its mRNA expression has been observed in various organs, indicating the activation of the extrinsic coagulation pathway [149]. The Noble–Collip drum model also exhibits a spontaneous thrombin burst measured by a thrombin generation assay due to the existence of circulating procoagulants. In addition, reduced levels of antithrombin result in systemic thrombin generation. These results therefore confirm the findings of the two previous clinical studies [90, 91]. Moreover, t-PA release driven by hypoperfusion of the endothelium leads to hyperfibrin(ogen)olysis. Meanwhile, there was no evidence of activated protein C-mediated shutdown of thrombin generation in the systemic circulation [93].

The tissue factor-induced DIC model demonstrated that a massive amount of tissue factor also induces DIC associated with fibrin(ogen)olysis without tissue hypoperfusion [106]. This suggests that trauma itself can give rise to DIC without tissue hypoperfusion, which supports the findings of a previous clinical study [68]. Tissue factor activation of coagulation leads to the generalized consumption of not only platelet and coagulation factors including fibrinogen but also the inhibitory feedback factors involved in controlling coagulation and fibrinolysis, namely, antithrombin and α2-plasmin inhibitor, respectively [90, 101, 106].

Management of DIC

The cornerstone of DIC management is specific and vigorous treatment of the underlying disorder, that is, injury itself and hemorrhagic shock [111, 150]. There are clear differences in the treatment of DIC with the fibrinolytic phenotype in the early phase of trauma and DIC with the thrombotic phenotype in the late phase of trauma. Management of the latter DIC type is the same as for typical DIC; anticoagulants, platelet and plasma substitution, and coagulation inhibitor concentrates have been proposed [111, 150]. Our discussion will follow with the treatment of fibrinolytic phenotype DIC through a novel approach.

After careful deliberation of "one concept and six considerations" for hemostatic changes dur-

ing the early phase of trauma proposed by the SSC on DIC of the ISTH [151], the initial care of the injured patient is essential, and guidelines on the management of major bleeding should be obeyed [152, 153]. DIC patients reach to the critical thresholds of platelet counts, PT, APTT, fibrinogen, and massive transfusion earlier than non-DIC patients. Furthermore, DIC patients are transfused with a higher volume of platelet concentrate, PRBC, fresh frozen plasma (FFP), and fibrinogen concentrate than non-DIC patients during the first 6 to 24 h after admission to the ED [154]. As such, substitution therapies with platelet concentrate, FFP, and fibrinogen concentrate are essential to maintain blood volume as well as normal platelet counts and function, adequate levels of coagulation factors, and endogenous anticoagulants including antithrombin and protein C [111]. Guidance for treatment of DIC published by the ISTH recommends the transfusion of FFP, which includes anticoagulant factors such as protein C, protein S, and antithrombin [150]. The use of agents that are capable of restoring dysfunctional anticoagulant pathways in DIC patients with sepsis has been extensively studied. However, there has been no study on the use of anticoagulant factor concentrates such as recombinant human thrombomodulin and antithrombin in trauma-induced DIC. Supranormal levels of anticoagulant factors foster bleeding. At present, FFP transfusion to maintain normal levels of protein C and antithrombin may be a reasonable strategy for treating DIC after trauma. We should be aware that anticoagulants are contraindicated for DIC with the fibrinolytic phenotype.

Tranexamic acid can reduce the risk of death in bleeding trauma patients [155] and should be given as early as possible because any delay in administration after trauma reduces its efficacy and may actually be harmful [156]. These studies provide the theoretical basis for antifibrinolytic therapy in DIC with the fibrinolytic phenotype in the early phase of trauma [111, 150]. Since the publication of the CRASH-2 results, however, there has been considerable discussion about how tranexamic acid should be used for bleeding patients in practice [157, 158]. Regarding the fibrinolytic spectrum, more discussion is needed in order to identify the real targets of tranexamic acid.

Trauma-Induced Coagulopathy and DIC

Trauma-induced coagulopathy (TIC) consists of a trauma itself-evoked primary pathology and exogenously induced secondary pathologies, such as anemia-, hypothermia-, acidosis-, and dilution-induced coagulopathy [159] (Table 14.2). The core disease state of TIC is the trauma "itself"-evoked primary pathology, namely, DIC. Like sepsis-induced coagulopathy, the clinical deterioration of TIC leads to DIC [160]. The SSC on DIC of the ISTH published a communication noting that TIC includes DIC, and if trauma is sufficiently severe, TIC progresses to DIC [161]. This is a reasonable statement and can be accepted. Figure 14.8 shows the relationships between DIC and TIC [161].

Table 14.2 Classification of trauma-induced coagulopathy

Trauma-induced coagulopathy
1. Physiological changes
Hemostasis and wound healing
2. Pathological changes
Trauma itself-induced primary pathology
Disseminated intravascular coagulation (DIC)
Activation of coagulation
Insufficient anticoagulant mechanisms
Increased fibrin(ogen)olysis (early phase)
Suppression of fibrinolysis (late phase)
Exogenously induced secondary pathologies that modify DIC
Anemia-induced coagulopathy
Dilutional coagulopathy
Hypothermia-induced coagulopathy
Acidosis-induced coagulopathy
Others

Detailed description is given in the text. Modified with permission [159]

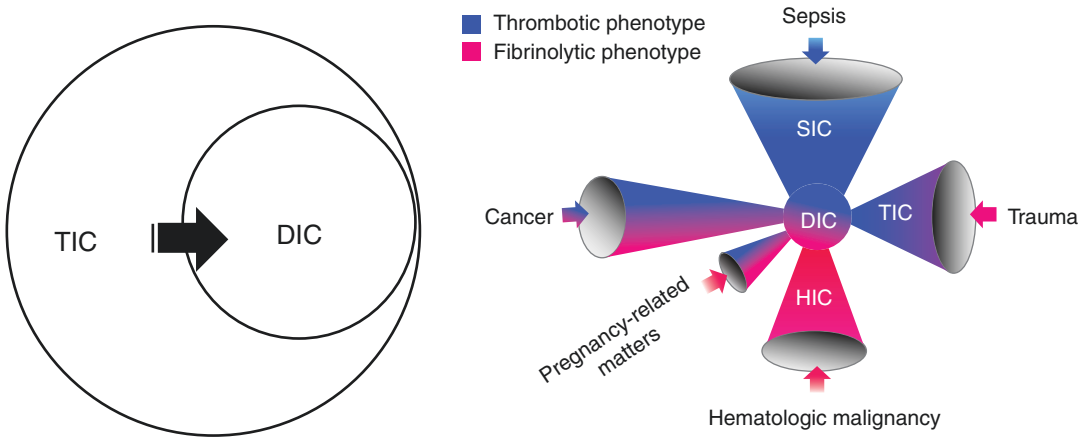


Fig. 14.8 Relationships between DIC and TIC. Left, TIC consists of trauma itself-induced primary pathology, i.e., DIC, and exogenously induced secondary pathologies (Refer to Table 14.2). Therefore, TIC includes DIC, and if trauma is sufficiently severe, TIC progresses to DIC (arrow). Right, TIC progresses to DIC just same as in the other fields such as sepsis-induced coagulopathy or hematologic malignancy-induced coagulopathy. DIC is a final dysregulated inflammatory and coagulofibrinolytic response to the insults. DIC disseminated intravascular coagulation, HIC hematological malignancy-induced coagulopathy, PC protein C, SIC sepsis-induced coagulopathy, sTM soluble thrombomodulin, TF tissue factor, TFPI tissue factor pathway inhibitor, TIC trauma-induced coagulopathy, TM thrombomodulin, t-PA tissue-type plasminogen activator. (Reprinted with modification with permission from [161])

Conclusion

The main pathophysiological mechanism of TIC is considered to be DIC. DIC in the early phase of trauma presents itself as a fibrinolytic phenotype, is associated with hyperfibrin(ogen)olysis and consumption coagulopathy, and contributes to massive hemorrhage. This type of DIC progresses to DIC with the thrombotic phenotype during the late phase of trauma and drives multiple organ dysfunction, leading to a poor outcome. To understand the pathogenesis and appropriate management of DIC, deep insights are needed into the interplay between innate immunity, inflammation, and coagulation and fibrinolysis.

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A L G r a w a n y

Part IV

Coagulation Assessment

15 Grading Clinical Coagulopathy and Predicting Massive Transfusion

Sophie Thorn and Marc Maegele

Abbreviations

A10	Clot amplitude at 10 minutes
ABC	Assessment of blood consumption
ACIT	Activation of coagulation and inflammation in trauma
aPTT	Activated partial thromboplastin time
AUROC	Area under the receiver operating characteristic curve
CA5	Clot amplitude at 5 minutes
CAT	Critical administration threshold
COAST	Coagulopathy of severe trauma
DMBT	Dynamic massive blood transfusion
ETS	Emergency room transfusion score
FAST	Focussed assessment and sonography in trauma
FFP	Fresh frozen plasma
GCS	Glasgow coma scale
INR	International normalised ratio
ISS	Injury severity score
MCF	Maximum clot firmness
MSI	Modified shock index
MT	Massive transfusion
MTP	Massive transfusion protocol
MTS	Massive transfusion score
PACT	Prediction of acute coagulopathy of trauma
PP/HR	Pulse pressure/heart rate
PRBC	Packed red blood cells
PROMMTT	Prospective observational multicentre major trauma transfusion
PT	Prothrombin time
PWH	Prince of Wales hospital
RABT	Revised assessment of blood transfusion
ROTEM	Rotational thromboelastometry
r-TEG	Rapid thromboelastography
SI	Shock index
TASH	Trauma-associated severe haemorrhage
TBSS	Traumatic bleeding severity score
TEG	Thromboelastography
TIC	Trauma-induced coagulopathy
TICCS	Trauma-induced coagulopathy clinical score
TR-DGU	TraumaRegister of the Deutsche Gesellschaft für Unfallchirurgie
TXA	Tranexamic acid

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Introduction

Uncontrolled haemorrhage and complicating trauma-induced coagulopathy (TIC) remain the most common cause of preventable death after

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trauma. Approximately one in every four bleeding trauma patients arrives to the emergency department having already developed a clinically significant laboratory coagulopathy [1]. TIC aggravates major haemorrhage with significant blood loss. Trauma patients are also major consumers of hospital blood product supplies: approximately 10% of severely injured patients receive at least one unit of blood product and trauma patients consume approximately 70% of blood products available to emergency departments [1, 2]. The highest mortality in patients who require massive transfusion (MT), which is most often defined as ≥10 units of blood products within 24 hours, occurs in the first 6 hours after injury [3]. The high mortality associated with massive transfusion is likely due to the development of coagulopathy and the subsequent occurrence of the 'lethal triad': coagulopathy, hypothermia and acidosis [4].

The best definition of massive transfusion is the topic of much discussion. The traditional definition of ≥10 units of packed red blood cells (PRBCs) within 24 hours misses patients who required blood products but died before receiving 10 units. An appropriate threshold for the number of units of blood products and the period of time in which they are administered is yet to be decided upon. The critical administration threshold (CAT) is defined as ≥3 units of PRBCs within the first hour and resuscitation intensity (RI), the total number of units of blood products, crystalloids or colloids administered within the first 30 minutes, of ≥4 are known alternatives to the conventional massive transfusion definition [5, 6]. A comparison between several definitions of massive transfusion validated on 1245 patients from the PROMMTT database is shown in Table 15.1 [7]. While mortality was highest in patients who received ≥10 units within 24 hours, by far more patients received 4 units or more in 4 hours, 6 units or more in 6 hours or 3 units or more in 1 hour and the mortality in these groups was almost as high. This corroborates the theory that the conventional definition is not sensitive enough to include all massively bleeding patients. RI and the CAT are limited by the possibility of missing patients in whom treatment with blood products is delayed due to misdiagnosis in the early phases of treatment or slow arrival of blood products. The alternate time frame options have a higher sensitivity for vulnerable exsanguinating patients at the expense of a lower specificity than the conventional definition.

Substantial progress has been made in reducing deaths due to trauma by instituting massive transfusion protocols (MTPs) at trauma centres [8]. MTPs give critical care physicians guidelines on the administration of blood products. The aim of an MTP is to have multiple units of blood products available quickly and in a safe and effective ratio, i.e. equal numbers of units of PRBC, fresh frozen plasma (FFP) and platelet concentrate [9]. Even with an MTP in place, any delay in the availability of blood products has dangerous consequences. The odds of mortality may increase by 5% for every minute spent waiting for requested blood products to arrive. An increased interval between activation of an MTP and arrival of the first blood products worsens the

Table 15.1 Definitions of massive transfusion

	≥10 PRBCs in 24 hours	≥4 PRBCs in 4 hours	≥6 PRBCs in 6 hours	≥3 PRBCs in 1 hour (CAT)	RI ≥ 4
N (%)	297 (23.9%)	599 (48.1%)	395 (31.7%)	570 (45.8%)	264 (21.2%)
PRBCs in 24 h (median [IQR])	16 [12–27]	8 [5–16]	12 [8–22]	9 [5–16]	8 [4–17]
24-hour mortality (n,%)	83 (27.9%)	119 (19.9%)	95 (24.1%)	130 (22.8%)	89 (24.5%)
3-day mortality (n,%)	118 (39.7%)	179 (29.9%)	135 (34.2%)	176 (30.9%)	112 (30.8%)

PRBC, packed red blood cells; *IQR*, interquartile range; *CAT*, critical administration threshold; *RI*, resuscitation intensity

patient's prognosis [8]. Similarly, a longer time period between the arrival of a traumatically injured patient and the decision to activate the MTP is associated with significantly higher mortality. Typically, the decision to activate an MTP is made by a physician based on knowledge and experience [10]. Most trauma centres do not use a strict protocol or prediction model to guide activation of an MTP.

Earlier identification of the severely injured patient requiring a massive transfusion benefits the patient and reduces mortality. It allows clinicians to rapidly address factors causing or exacerbating haemorrhage, manage complications of major haemorrhage such as coagulopathy, hypothermia and acidosis, activate an MTP prior to or shortly after arrival of the patient at the hospital, and mobilise resources to best manage the exsanguinating patient [1]. Accurate prediction of patients requiring massive transfusion can also reduce the volume of blood products that are ultimately administered to the patient by allowing effective ratios to be given [10]. Early, aggressive administration of clotting factors, such as in FFP or cryoprecipitate, can reduce the effect of, or even prevent, TIC and thus increase survival [9, 11, 12]. Early risk stratification of vulnerable patients allows the medical team to prepare for worst-case scenarios and provide appropriate and targeted treatment.

Conventional Coagulation Tests for Diagnosing Trauma-Induced Coagulopathy and Predicting Massive Transfusion

Historically, trauma-induced coagulopathy has been identified and diagnosed using conventional coagulation tests such as prothrombin time (PT) or international normalised ratio (INR), activated partial thromboplastin time (aPTT) or fibrinogen level. A summary of results of conventional coagulation assays which may indicate coagulopathy is providerd in Table 15.2. These tests provide an overview of the functioning of a complex pathway leading to clot formation. The INR or PT assesses the function of the extrinsic pathway and aPTT assesses the intrinsic pathway; these together lead to the initiation of thrombin formation. There is limited information in these tests regarding coagulation processes after thrombin is formed [13]. They are by no means a complete picture of the state of a patient's coagulation and give insufficient details to guide specific treatment in a severely injured patient [14].

There is a lack of consistency among published studies as to the appropriate INR and aPTT threshold to diagnose TIC. Many studies define TIC as INR >1.2 [15, 16] while others state that significant coagulopathy only occurs at INR >1.5 [17, 18]. INR may be more accurate than aPTT in identifying coagulation defects in vivo in trauma patients [19]. Other studies show that INR- and aPTT-based diagnosis is inferior to thromboelastography (TEG)- or rotational thromboelastometry (ROTEM)-based diagnosis [20].

Table 15.2 Results of conventional coagulation assays and viscoelastic testing which may indicate coagulopathy

Assays	Definition of coagulopathy
Conventional assays	
INR	≥1.2
aPTT (sec)	>6
Platelets ($\times 10^9$/L)	<100
Fibrinogen (g/L)	<150
ROTEM	
EXTEM CT (sec)	>80
EXTEM CFT (sec)	>159
EXTEM MCF (mm)	<50
INTEM CT (sec)	>240
INTEM CFT (sec)	>110
INTEM MCF (mm)	<50
FIBTEM MCF (mm)	<9
TEG	
Rapid-TEG ACT (sec)	>140
R time (min)	>10
K time (min)	>3
α angle (degrees)	<53
MA (mm)	<50
LY30 (%)	>3

INR, international normalised ratio; *aPTT*, activated partial thromboplastin time; *CT*, clotting time; *CFT*, clot formation time; *MCF*, maximum clot firmness; *ACT*, activated clotting time; *R time*, time to initial fibrin formation; *K time*, time to reach 20 mm clot strength; *α angle*, measure of speed of clot formation; *MA*, maximum amplitude; *LY30*, clot lysis at 30 minutes after MA

INR results do have some accuracy in identifying requirement of MT. [21, 22] In retrospective cohort analyses, patients with deranged INR have been significantly more likely to receive an MT, and indeed INR >1.5 is often the most strongly correlated variable when multiple regression analysis is performed [23]. Unfortunately, the utility of implementing INR or aPTT as triggers for MTP activation is limited by the turnaround time of most conventional coagulation tests. As the time to administering the first blood products is so critical in determining mortality, it is not acceptable as common practice to delay activation of the MTP by 40–60 minutes while waiting for laboratory results to return. Point-of-care INR testing has been developed but currently may not correlate sufficiently well with conventional tests to be relied upon [24].

Other biochemical markers of bleeding and coagulopathy have also been associated with the requirement of massive transfusion. Base deficit correlates well with INR and aPTT results and is available much sooner as part of a blood gas analysis [25]. A base deficit of >2 in combination with systolic hypotension and a high injury severity score was strongly associated with coagulopathy [26] and thus could be used to guide blood product transfusion including MT. In the same trial, lactate was not significantly correlated with derangements in conventional coagulation tests or evidence of major haemorrhage, despite being a marker of blood loss.

Predicting Trauma-Induced Coagulopathy and Massive Transfusion Using Viscoelastic Testing

Viscoelastic testing, which includes TEG and ROTEM, provides information on how quickly clot formation is initiated, the rate of clot growth, the strength of the clot and the process of clot breakdown [27]. Several studies have investigated the efficacy of using viscoelastic tests to diagnose TIC and prompt the provision of blood products [28–30]. ROTEM results are typically available, at least in part, sooner than conventional coagulation tests and also can guide the administration of selected blood products to remedy specific coagulation defects [31]. A summary of results of viscoelastic testing assays (ROTEM and TEG) which may indicate coagulopathy is shown in Table 15.2.

TEG assays give several distinct pieces of information regarding coagulation: activated clotting time (ACT) describes the time taken for clot formation to commence, in seconds; α angle is the rate of clot strength increase; K time is the time taken for a clot to reach 20 mm in size; maximum amplitude (MA) is the measurement of the clot at its maximum, in mm; and LY30 is the percentage of the clot which has undergone lysis 30 minutes after maximum clot strength [32]. Rapid TEG (r-TEG) involves the addition of tissue factor to the blood sample to expedite clot formation, leading to earlier results [33]. R-TEG performs well at quickly identifying patients who require massive transfusion [34, 35]. Each of the results suggests need for a particular treatment: patients with prolonged ACT require PRBCs; those with decreased α angle need cryoprecipitate and FFP; decreased MA is managed with platelet transfusion and increased LY30 may be treated with tranexamic acid [32]. On r-TEG, MA and α angle predict MT with AUCs of ≥0.80 [34, 36]. The addition of tissue plasminogen activator (tPA) to blood samples before performing TEG assays aims to mimic the endogenous release of tPA which is thought to cause hyperfibrinolysis following trauma. LY30 had an AUC of 0.86 for MT on this modified tPA-TEG [37]. The time to availability of LY30 and MA results was decreased due to the addition of tPA, and MT was predicted accurately using several different definitions [38].

Four assays are possible using ROTEM: EXTEM investigates the function of the extrinsic pathway; INTEM assesses the intrinsic pathway; FIBTEM describes the contribution of fibrinogen to clot formation; and APTEM evaluates for the presence of hyperfibrinolysis [39]. Derangements in INTEM maximum clot firmness (MCF) and EXTEM clot amplitude at 5 minutes (CA5) results have been most strongly correlated with a requirement of massive transfusion, as well as the presence of coagulopathy diagnosed on conven-

tional coagulation tests [15]. FIBTEM MCF and clot firmness at 10 minutes (A10) were also highly predictive of MT, with AUCs of 0.84 and 0.83, respectively [32, 40].

Prediction Scores for Trauma-Induced Coagulopathy

Expediting the diagnosis of coagulopathy in the severely injured patient will help guide administration of coagulopathy-targeted treatment, such as tranexamic acid (TXA), in addition to blood products including massive transfusion. Prediction models that combine clinical variables can produce a forecast of whether or not any individual patient will be diagnosed with or suffer consequences of coagulopathy. Four prediction models for TIC have been developed, some of which have been validated, but none are in widespread use.

The Coagulopathy of Severe Trauma (COAST) Score

The COAST score was developed and validated on a single centre Australian cohort of 2905 patients in 2011 and uses five variables that are available before arrival at the hospital [18]. The variables are vehicular entrapment (associated with prolonged extrication time), prehospital systolic blood pressure, prehospital temperature, chest decompression and abdominal or pelvic injury (Table 15.3). The outcome, TIC, was defined as INR >1.5 or aPTT >60s. At a threshold of ≥3 points, the COAST score had a sensitivity of 60% and specificity of 96.4%. The score has been externally validated on a Belgian trauma cohort of 133 patients (with sensitivity 80% and specificity 96%) and on 15,370 patients from the TraumaRegister for the Deutsche Gesellschaft für Unfallchirurgie (TR-DGU) with sensitivity 21.6% and specificity 94.2% [41, 42].

Table 15.3 The Coagulopathy of Severe Trauma Score [18]

Variable	Value	Points
Entrapment	Yes	1
Systolic blood pressure	<100 mmHg <90 mmHg	1 2
Temperature	<35 °C <32 °C	1 2
Chest decompression	Yes	1
Abdominal or pelvic content injury	Yes	1

The Bayesian Network Model

This complex prediction model was developed on the Activation of Coagulation and Inflammation in Trauma (ACIT) study cohort in the UK and validated in the UK and Germany [43]. It utilises 14 weighted variables identified through artificial intelligence modelling to predict the presence or absence of coagulopathy (Table 15.4). TIC was defined as prothrombin time ratio (INR) >1.2. The Bayesian network model requires input of the variables into an online calculator which gives a numerical likelihood of developing TIC and also a dichotomous (yes/no) result so that the score may be clinically useful. At the cut-point chosen, sensitivity for laboratory-diagnosed TIC was 90% and specificity was 92%. The area under the receiver operating characteristic (AUROC) curve was 0.964.

Table 15.4 The Bayesian Network Model [43]

Variable	Value
Heart rate	Online value
Systolic blood pressure	Online value
Temperature	<34 °C or ≥ 34 °C
Haemothorax	Yes
FAST scan	Positive
Unstable pelvic fracture	Suspected
Long bone fracture	Suspected
Glasgow Coma Scale	Online value
Lactate	Online value
Base deficit	Online value
pH	Online value
Mechanism of injury	Blunt or penetrating
Energy of injury	High or low velocity
Volume of intravenous fluid administered	<500 mL 500–2000 mL >2000 mL

The Trauma-Induced Coagulopathy Clinical Score (TICCS)

The TICCS is a simple score using three basic variables that was developed on a small Belgian cohort of 82 patients [44] and validated externally on a 33,385 patients from the TR-DGU [45]. The score combines variables of triage destination, systolic blood pressure and distribution of injuries to predict coagulopathy (Table 15.5). The maximum score is 18 points, although all patients registered on the TR-DGU are admitted to a resuscitation room, so all cases on the validation scored at least 2 points. The outcome investigated was coagulopathy based on 1) TEG/ROTEM results, 2) INR >1.3 or 3) fibrinogen <1.5 g/L, plus other signs of bleeding-related outcomes. In the development study, a cut-off of TICCS ≥10 points was 100% sensitive and 96% specific. The validation study used any blood transfusion as outcome of interest, and a cut-off of TICCS ≥12 points had a positive predictive value of 48.4% and negative predictive value of 89.1%.

The Prediction of Acute Coagulopathy of Trauma (PACT) Score

The PACT score was developed on a prospective cohort study in Washington, USA, then subsequently externally validated on the Oregon Trauma Registry and substantially altered [46, 47]. The resulting score uses six weighted variables for predicting the presence of coagulopathy. A total of 1963 patients were evaluated in the development study and TIC was defined as INR >1.5. A threshold of ≥196 predicted TIC with 73.1% sensitivity and 73.8% specificity (Table 15.6).

Common themes between these four published prediction models in addition to reports describing individual associated variables are the presence of systolic hypotension (or high shock index) and evidence of high injury severity [17, 18, 43, 44, 47]. Markers of severe injury included chest decompression, pelvic or long bone fracture, injury mechanism and energy, admission to the resuscitation room and a high Injury Severity Score (ISS). These are effective predictive measures because they are intrinsically involved in the mechanism of the development of trauma-induced coagulopathy [48]. The volume of crystalloid fluid administered is also associated with the presence of coagulopathy, but likely represents a dilutional coagulopathy occurring later rather than one developing as a direct result of the injury [17].

Most of the above TIC prediction scores have not been formally validated to predict MT. In the COAST score validation on the TR-DGU, MT was a secondary outcome and significantly more common among cases with positive COAST scores (15.3%) versus cases with negative COAST scores (1.6%) [42]. These scores were not designed to predict and prompt MT; rather, they were designed to guide other management techniques, such as the early administration of tranexamic acid or the initiation of damage control resuscitation [18, 44, 46]. Importantly, these scores were also designed to stratify patients for inclusion in clinical trials for treatments of TIC

Table 15.5 The Trauma-Induced Coagulopathy Clinical Score [44]

Variable	Value	Points
Triage destination	Resuscitation room	2
	General emergency room	0
Systolic blood pressure	<90 mmHg	5
	≥90 mmHg	0
Significant injuries	Head/neck	1
	Left upper extremity	1
	Right upper extremity	1
	Left lower extremity	1
	Right lower extremity	1
	Torso	2
	Abdomen	2
	Pelvis	2

Table 15.6 The Prediction of Acute Coagulopathy of Trauma Score [47]

Variable	Value	Points
Prehospital shock index	>1	90
Age	Years	1 point/year
Traffic mechanism	No	50
Glasgow Coma Scale	–	Points below 15
Prehospital cardiopulmonary resuscitation	Yes	120
Prehospital intubation	Yes	50

that are not limited to massive transfusion [49, 50]. For the specific prediction of massive transfusion requirement, several predictive models have been developed. These are discussed below.

Civilian Prediction Scores for Massive Transfusion

Many models have been developed to predict the requirement for massive transfusion in severely injured patients. MT is defined as ≥10 units of PRBCs within 24 hours unless stated otherwise. A suggested alternative definition is ≥5 units of PRBCs within 4 hours [48]. Population characteristics differ between the score development and validation studies; thus there are different proportions of cohorts who receive MT.

The Assessment of Blood Consumption (ABC) Score

The ABC score was developed for the prediction of massive transfusion requirement by Nunez in 2009 [2]. It comprises four non-weighted variables available quickly upon arrival in the emergency department: penetrating mechanism, positive focussed assessment and sonography in trauma (FAST), arrival systolic blood pressure and arrival heart rate (Table 15.7). Each variable is dichotomous, and patients are awarded 1 point for presence of the risk factor or 0 point for absence. On the development cohort of 596 patients, of whom 74 (12.6%) had an MT, a score of 2 or greater was 75% sensitive and 86% specific for requiring MT.

Since its development, the ABC score has been tested and validated several times on a variety of cohorts. Sensitivity of the score ranged between 47% and 90% on various cohorts, and the area under the receiver operating characteristic (AUROC) curve ranged from 0.68 to 0.86 [51–58]. The score has also been analysed in conjunction with blood lactate levels to evaluate their combined predictive ability. The ABC score alone had a sensitivity of 82% and specificity 34% but combined with blood lactate >4 mmol/dL the sensitivity and specificity rose to 92% and 42%, respectively [56]. The ABC score appears to be less accurate among older patients than among younger, but this is consistent among two other scores that were tested on the same cohort [55]. The largest external validation of the ABC score to date was on 5147 patients from the TR-DGU. On this cohort, an ABC score of ≥2 was 76.1% sensitive and 70.3% specific for MT requirement, with an AUROC of 0.763 [53].

A study published in 2018 compared the frequency of MTP activation and the mortality rate among hypotensive trauma patients before and after implementation of the ABC score to trigger MTP activation [59]. The number of MTP activations increased sixfold (from 15 to 93), and balanced, ratio-based blood product provision also increased dramatically. Mortality among hypotensive patients decreased from 41.6% to 18.9% post-implementation. This successful implementation demonstrates the utility of a simple, quick and effective score to predict MT and thus trigger activation of the MTP. The major benefits of this score are the ease and speed with which it can be calculated, because of not relying on any laboratory tests or scans. Some of the limitations are that it usually cannot be calculated before arrival at the hospital, because of the FAST scan, and that the accuracy of the score is quite inconsistent among different populations. The ABC score remains the most thoroughly validated and most widely used massive transfusion prediction score.

Table 15.7 The Assessment of Blood Consumption Score [2]

Variable	Value	Points
Penetrating mechanism	Yes	1
Systolic blood pressure	≤ 90 mmHg	1
Heart rate	≥ 120 bpm	1
FAST scan	Positive	1

Trauma-Associated Severe Haemorrhage (TASH) Score

The TASH score was developed through multiple logistic regression of likely predictive variables on a cohort of 4527 patients from the TR-DGU and

was published in 2006 [60]. It was internally validated on an equivalent cohort from the TR-DGU. The resulting score comprises seven weighted variables which must be inserted into the formula: $p = 1/[1 + \exp(4.9 - 0.3 * TASH)]$ to calculate the probability of a patient requiring an MT (Table 15.8). At a threshold of TASH >16, MT was observed to occur 50% of patients. The variables involved in the score are haemoglobin, base excess, systolic blood pressure, heart rate, free intra-abdominal fluid (e.g. noted on FAST), injury to the extremities (i.e. clinically unstable pelvic fracture or open/displaced femur fracture) and male sex. A maximum score of 28 points is possible. The predicted rate of MT was 13.9% and the observed rate on the initial population was 14.1%.

The TASH score was revalidated over a four-year period of the TR-DGU and an updated logistic function was published: $p = 1/[1 + exp(5.4 - 0.3 * TASH)]$ in order to attain appropriate accuracy [61]. The threshold for 50% likelihood of MT was modified to 18 points. With the new formula, the score had an AUROC of 0.905 and the predicted rate of MT (8.7%) closely resembled the observed rate (8.4%). Furthermore, an attempt was made to improve the score by including other variables such as INR, lactate, pH or mechanism of injury, which did not improve the accuracy of the TASH score.

Table 15.8 The Trauma-Associated Severe Haemorrhage Score [60]

Variable	Value	Points
Haemoglobin	<7 g/dL	8
	<9 g/dL	6
	<10 g/dL	4
	<11 g/dL	3
	<12 g/dL	2
Base excess	< −10 mmol/dL	4
	< −6 mmol/dL	3
	< −2 mmol/dL	1
Systolic blood pressure	<100 mmHg	4
	<120 mmHg	1
Heart rate	>120 bpm	2
FAST scan	Free intra-abdominal fluid	3
Extremities	Unstable pelvic fracture	6
	Open/dislocated femur fracture	3
Male patient	Yes	1

The TASH score has since been externally validated and studied extensively. A further validation on the TR-DGU used a cut-off of ≥9 points for MT and achieved a higher sensitivity of 84.4%, at the expense of the specificity which was decreased to 78.4% [53]. External validations performed at other trauma centres found an AUROC between 0.51 and 0.88 [54, 58, 62, 63]. The TACH score was found to be not predictive in a cohort of rural patients, with an AUROC of 0.51 [62]. The performance of the TASH score was also evaluated on a cohort of obese and non-obese patients, as MT is more common in obese patients [64]. The score performed similarly for obese and non-obese patients with an AUROC of 0.93 and 0.94, respectively. The TASH score was found to be much more accurate on a cohort of young patients (sensitivity 81% and specificity 80%) than on a cohort of older patients (sensitivity 56% and specificity 76%) [55].

The high overall accuracy and reliability of the TASH score is a major advantage. Four of the seven variables (systolic blood pressure, heart rate, gender and injuries) can be assessed immediately upon arrival, while the other three (haemoglobin, base excess and free intra-abdominal fluid) require diagnostic tests. This factor and the fact that it cannot be easily remembered and calculated on the go are downfalls of the score: a computer or chart is needed to calculate the TASH score. A possible benefit of the score is the flexibility with the threshold for MT; it would be possible for trauma centres to validate the score on their cohort, retrospectively or prospectively, and ascertain which threshold may be appropriate locally for MTP activation.

Prince of Wales (PWH)/Rainer Score

The PWH score was developed on 1891 patients at a trauma centre in Hong Kong and published in 2011 by Rainer et al. [65]. The score uses eight weighted variables to predict the need for MT, defined as ≥10 units of PRBCs within 24 hours or ≥ 8 units within 12 hours. Ninety-two (4.9%) patients in this cohort received MT. The variables used are systolic blood pressure, Glasgow Coma

Scale (GCS), heart rate, displaced pelvic fracture, CT or FAST evidence of intra-abdominal bleeding, base deficit and haemoglobin (Table 15.9). A cut-off of ≥6 points was used to predict MT, and it was 31.5% sensitive and 99.7% specific. The PWH has a maximum of 10 points which can be attained with a positive result in each of the variables or with just a haemoglobin ≤7 g/dL, which scores 10 points.

The PWH score has undergone several large validations. A validation on a cohort of 5147 patients on the TR-DGU demonstrated an AUROC of 0.86, sensitivity 80.6% and specificity 77.7% [11]. The PWH score performed with sensitivity 36.9% and specificity 97.1% on an Australian cohort of 1234 patients [66]. More recently, the score was validated on a multicentre cohort from Hong Kong of 4991 patients and had a sensitivity of only 3.4% and specificity 99.1% [63]. When another validation utilised the most accurate cut point for predicting MT, PWH ≥2, sensitivity was 92.9% and specificity 59.8% [58]. Similarly to other scores which were validated on young and older populations, the PWH score was less accurate on an older cohort: the sensitivity decreased from 80% to 68% (at a cut-off of ≥2), while the specificity remained stable [55].

The low sensitivity of the PWH score markedly affects its efficacy as physicians must maintain a high index of suspicion in patients who do not meet criteria for MTP activation [65]. A further consideration is the weighting of haemoglobin ≤7 g/dL which is able to trigger the MTP in isolation. This prediction model also requires some information which is only available upon arrival to the hospital, after blood tests and scans have been performed.

Table 15.9 The Prince of Wales/Rainer Score [65]

Variable	Value	Points
Systolic blood pressure	<90 mmHg	3
Glasgow Coma Scale	≤8	1
Heart rate	≥120 bpm	1
Displaced pelvic fracture	Yes	1
CT or FAST scan	Positive	2
Base deficit	>5 mmol/L	1
Haemoglobin	≤7 g/dL 7.1–10 g/dL	10 1

The Vandromme Score

Vandromme et al. developed and internally validated a prediction model on a civilian cohort of 6639 patients [67]. Of the included patients, 158 (2.4%) received an MT. Five variables were identified which were included in the non-weighted prediction model. Blood lactate, heart rate, haemoglobin, systolic blood pressure and INR were included (Table 15.10). A good balance between sensitivity and specificity proved difficult to attain; the best results were with a threshold of ≥3 points, which produced a sensitivity of 53.4% and specificity 97.6%. The AUROC was 0.9.

The Vandromme score was validated on a large cohort from the TR-DGU and had a cut-off of ≥2 points assigned, to achieve the best balance of sensitivity and specificity [11]. The AUROC was 0.84, the sensitivity 78.9% and the specificity 76.2%. This model shares the limitation with a previously described score that INR takes time to measure and be made available. The other four variables are available quickly upon arrival with the use of point-of-care tests and clinical examination.

Seven predictive scores were validated simultaneously on the TR-DGU to compare their accuracy [1]. The TASH score, ABC score, Baker model, PWH score, Vandromme score, Schreiber score and Larson score were validated on 7042 civilian patients. Their performance is summarized in Fig. 15.1.

The Shock Index

The shock index (SI) is the ratio of heart rate to systolic blood pressure, which typically sits between 0.5 and 0.7 in healthy adults. This has

Table 15.10 The Vandromme Score [67]

Variable	Value	Points
Blood lactate	≥5 mmol/L	1
Heart rate	>105 bpm	1
Haemoglobin	≤11 g/dL	1
INR	>1.5	1
Systolic blood pressure	<110 mmHg	1

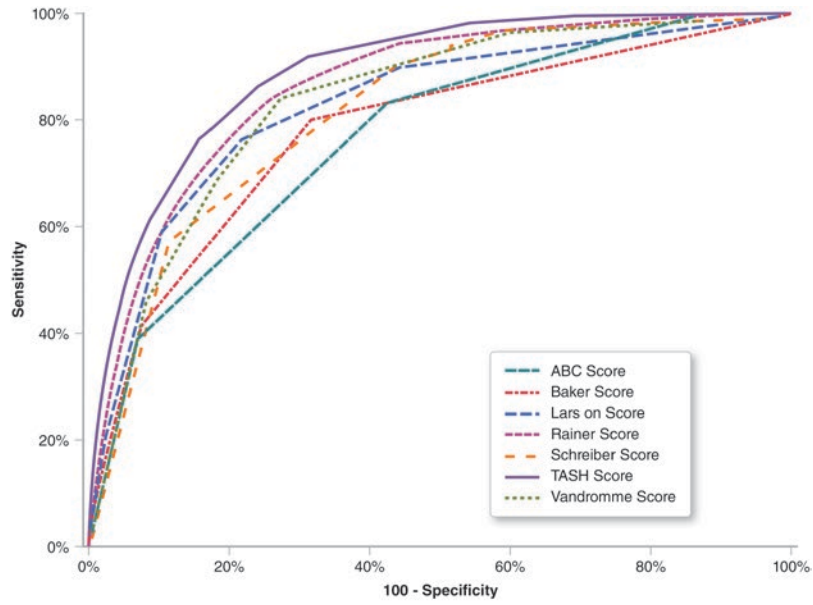


Fig. 15.1 Results of a validation of seven massive transfusion prediction scores on 7042 patients on the TR-DGU. The scores are compared based on the area under the receiver operating characteristic curve. (Modified from Maegele, et al. [1])

Score/Algorithm	AUROC	Interval
ABC	0.779	0.758-0.800
Baker	0.768	0.749-0.787
Larson	0.832	0.816-0.848
PWH(Rainer)	0.858	0.844-0.872
Schreiber	0.805	0,787-0.823
TASH	0.890	0.878-0.902
Vandromme	0.847	0.831-0.862

been considered to be a useful measure of haemodynamic stability, particularly as many trauma patients with solely internal bleeding maintain normotension initially after injury. Vandromme in 2011 was the first to assess the accuracy of SI for predicting MT in trauma patients [68]. Of the 8111 included patients into the study, 276 (3.4%) required an MT. Increases in SI, particularly >0.9, were proportionally associated with increased probability of MT. At SI >1.3, likelihood of MT was >20%. Increasing SI was also associated with higher injury severity scores and higher mortality. A subsequent validation of SI on a cohort from the TR-DGU demonstrated a similar trend in increasing blood product transfusion requirements as SI increases [69]. A comparison between prehospital SI and initial SI on arrival to a trauma centre demonstrated that prehospital SI was more sensitive (57.8% vs 52.2%) but less specific (83.0% vs 93.1%) for MT. [70] Applying various formulae to combine and manipulate these numbers yielded few clinically useful results.

Several further validations have used different cut-offs to predict MT; however, each study had a threshold between SI ≥0.9 and SI ≥1.0. Using their individually determined thresholds, AUROC ranged from 0.76 to 0.83 [57, 58, 71–73]. Alternatives to the simple SI include the modified shock index (MSI): a ratio of heart rate to mean arterial pressure ([2 ∗ *diastolic blood pressure* + *systolic blood pressure*]/3), pulse pressure to heart rate ratio (PP/HR) and age shock index (shock index multiplied by the patient's age) [71, 73]. The PP/HR predicted MT with an AUROC of 0.77 at a threshold of PP/HR <0.443 [73]. The

MSI performed most accurately at a cut-off of MSI >1.15 with a sensitivity and specificity of 61.5% and 82.3%, respectively. The age shock index at a threshold of >36.95 performed with sensitivity 54.2% and specificity 72.3% [71].

The shock index is certainly the simplest predictive method developed thus far: it requires only two variables which can be obtained before or immediately upon arrival with no special tests. The recommended threshold for deeming a patient 'at risk' for massive transfusion is SI ≥0.9; however, this is not a very sensitive predictor. A further limitation is that an increasing shock index may not be specific to traumatic haemorrhage.

$$\text{Shock index} = \frac{\text{Heart rate}(\text{bpm})}{\text{Systolic blood pressure}(\text{mmHg})}$$

Emergency Room Transfusion Score (ETS)

The ETS was initially developed on a cohort of 1103 patients from a trauma centre in Essen, Germany, to predict the need for any blood transfusions [74]. On the development cohort, 116 (10.5%) patients required blood transfusions. The score involves six weighted variables which are assigned points up to a maximum of 9.5 points (Table 15.11). The variables are age, admission (from scene or other hospital), trauma mechanism (traffic accident or fall from height > 3 m), systolic blood pressure, abdominal ultrasound result and stability of pelvis on clinical exam. An ETS >3 points puts a patient in the high-risk category, in which 34.6% of patients received an urgent blood transfusion. In the low- and intermediate-risk groups, only 1.6% and 8.5%, respectively, of patients received transfusions. A subsequent internal validation on 481 patients demonstrated a sensitivity of 84.2% at the cut-off of >3 points and a specificity of 92.5% [75].

In 2019, the ETS was validated externally along with five other scores to assess its ability to predict MT [58]. The most effective cut-point for MT was found to be >5 points, with which a sensitivity of 95% and specificity of 60.8% were attained. It had an AUROC of 0.85, which made it the most accurate score validated in this study. The ETS is a simple score that can be calculated quickly without the need for blood tests but requires clinical examination by a clinician and a FAST or CT scan upon arrival.

Table 15.11 The Emergency Room Transfusion Score [74]

Variable	Value	Points
Age	0–20 years 20–60 years >60 years	0 0.5 1.5
Admission	From scene From other hospital	1 0
Mechanism	Traffic accident Fall from height > 3 m	1 1
Systolic blood pressure	>120 mmHg 90–120 mmHg 0–90 mmHg	0 1.5 2.5
Abdominal ultrasound	No free fluid Free fluid	0 2
Pelvis on clinical exam	Stable Unstable	0 1.5

Massive Transfusion Score (MTS)

The Prospective Observational Multicentre Major Trauma Transfusion (PROMMTT) study cohort was used to design and develop the MTS [22]. A total of 1245 patients were included in this development study and MT was administered to 297 (23.9%). The variables involved in the score are INR, systolic blood pressure, haemoglobin, base deficit, heart rate, penetrating mechanism and positive FAST (Table 15.12). A derived result was produced for patients who did not have a FAST scan because they were in extre-

Table 15.12 The Massive Transfusion Score [22]

Variable	Value	Points
INR	>1.5	1
Systolic blood pressure	<90 mmHg	1
Haemoglobin	<11 g/dL	1
Base deficit	≥6	1
Heart rate	≤120 bpm	1
Penetrating injury	Yes	1
Positive FAST scan	Yes	1

mis and went straight to the operating theatre for diagnostic laparotomy– this was awarded equal points as a positive FAST if intra-abdominal fluid was found. The authors chose not to weight the variables. The Sensitivity was 85% and the specificity 41% when FAST was included in the score.

A secondary analysis was performed to predict MT at 6 hours (i.e. ≥10 units of PRBCs within 6 hours). Deranged INR, base deficit and haemoglobin were the most strongly correlated variables.

More recently, authors from the same institution published the Revised MTS, which did not include heart rate, positive FAST or penetrating mechanisms as predictors. This new score had a better AUROC: 0.72 vs 0.60 [76]. The authors supported removing these elements as FAST scans are dependent on the user and can be inaccurate due to user error or inexperience; heart rate may no longer be a reliable measure in the aging trauma population, many of whom are taking medications and have pre-existing conditions which affect their heart rate; and penetrating injuries are simply not very common among civilian trauma populations.

The Traumatic Bleeding Severity Score (TBSS)

The TBSS was developed on 119 patients and internally validated on 113 patients from a single trauma centre [77]. Of the included patients, 62 (52.1%) received an MT. The score combines five weighted variables: patient's age, systolic blood pressure after rapid infusion of 1000 mL normal saline, number of positive FAST regions, presence and type of pelvic fracture and blood lactate concentration (Table 15.13). A certain number of points is assigned to each variable and these numbers are added together to a maximum of 57 points. A cut-off of 15 points was correlated with MT with 97.4% sensitivity and 96.2% specificity. The AUROC was 0.985. There was a subsequent internal validation on a later cohort with 32.2% MT rate [78]. The AUROC for this validation on 151 severely injured patients was 0.97.

Table 15.13 The Traumatic Bleeding Severity Score [77]

Variable	Value	Points
Age	≥60 years	6
	<60 years	0
Systolic blood pressure	<90 mmHg	12
	90–100 mmHg	8
	100–110 mmHg	4
	≥110 mmHg	0
FAST scan	Pericardium	3
	Right thorax	3
	Left thorax	3
	Perihepatic	3
	Perisplenic	3
	Pelvic	3
Pelvic fracture	Type A	3
	Type B	6
	Type C	9
Lactate concentration	≥7.5 mmol/L	12
	5–7.5 mmol/L	8
	2.5–5 mmol/L	4
	<2.5 mmol/L	0

The TBSS is more complex than some of the MT prediction scores discussed above; however, a smartphone application has been developed to speed up calculation and hence expedite appropriate activation of an MTP. The cohort upon which the TBSS was developed had a markedly older mean age (57.6 years, compared with 39.2 years in the TASH development cohort and 40 years in the ABC development cohort); thus, the impact of age was recognised.

Code Red

A 'code red' is the term used in the UK for sending a prehospital request for the hospital to activate the MTP in preparation for the arrival of an exsanguinating patient [79]. Weaver et al. aimed to assess whether three criteria were accurate for predicting MT: suspicion or evidence of active haemorrhage (from clinical examination and findings alone), systolic blood pressure and failure to respond to a fluid bolus (Table 15.14). During this investigation, 126 code red activations occurred, of which 115 (91%) patients received blood products upon arrival. Of these patients, 46 (40%) received an MT. A subsequent multicentre validation included 53 code red

Table 15.14 UK Code Red [79]

Variable	Value
Suspicion or evidence of active haemorrhage	Yes
Systolic blood pressure	<90 mmHg
Failure to respond to fluid bolus	Yes

activations, 47 (89%) of whom received blood products while six (11%) received MT [80].

This model was not created to predict MT, but rather any blood product transfusion. The low specificity for MT compared with the high specificity for any blood transfusions speaks to this. Additionally, the non-specific criterion of 'suspicion or evidence of active haemorrhage' does not lend itself well to widespread implementation of this score. One advantage of the score is the ability to calculate it quickly, prior to arrival at the hospital to expedite the provision of blood products.

The Dynamic Massive Blood Transfusion (DMBT) Score

The DMBT score was established in response to the PWH score in Hong Kong [63]. A total of 4991 patients were included in a multicentre development study and there were 166 (3.3%) patients requiring MT. Predictor variables that were included in the score were systolic blood pressure, heart rate, haemoglobin with subsequent haemoglobin drop, INR, base deficit, unstable pelvic fracture and evidence of haemoperitoneum on FAST or CT (Table 15.15). Variables were weighted according to the OR conferred by them and the maximum score possible was 21. At a cut-off of ≥6, sensitivity was 78.2% and specificity was 89.2%. The AUROC was 0.907.

Table 15.15 The Dynamic Massive Blood Transfusion Score [63]

Variable	Value	Points
Systolic blood pressure	≤90 mmHg	2
Heart rate	≥120 bpm	2
Haemoglobin (g/dL)	Initially >10 and drop <2 Initially 8.1–10 or drop 2–3.9 Initially ≤8 or drop ≥4	0 2 4
INR	≥1.3	2
Base deficit	<5 5–9.9 ≥10	0 2 5
Unstable pelvic fracture	Yes	4
Evidence of haemoperitoneum on FAST/CT scan	Yes	2

Table 15.16 The Revised Assessment of Bleeding and Transfusion Score [81]

Variable	Value	Points
Penetrating injury	Yes	1
FAST	Positive	1
Shock index	>1	1
Pelvic fracture	Yes	1

The Revised Assessment of Bleeding and Transfusion (RABT) Score

The RABT score follows and alters the ABC score in an attempt to improve on its sensitivity and specificity [81]. It utilises shock index (rather than heart rate and blood pressure as separate variables), positive FAST and penetrating mechanism, as well as the new addition of pelvic fracture (Table 15.16). The study cohort was 380 patients, of whom 102 (26.8%) received MT. A threshold of ≥2 points was chosen for its sensitivity of 84% and specificity of 77%, with the score attaining an AUROC of 0.828. The RABT was more accurate than the ABC score on this cohort; ABC had 39% sensitivity, 72% specificity and an AUROC of 0.617. This score has not been externally validated to date.

The Baker Model

The Baker model was the first transfusion prediction score, having been developed in a US level 1 trauma centre and published in 2001 [82]. This model utilises four clinical variables available upon arrival at the hospital: systolic blood

pressure, heart rate, GCS and 'high-risk injury', which included chest trauma, abdominal injuries with peritonism, death of a passenger in the same vehicle, ejection from a vehicle and penetrating injuries to the torso (Table 15.17). The presence of all four risk factors was associated with a 100% transfusion rate, while zero risk factor was associated with a 2% transfusion rate. This model does not look directly at predicting massive transfusion; however, a large proportion of the cohort did receive a massive transfusion. The rather vague and difficult to remember variable of 'high-risk injury' is a limitation to the implementation of this score.

Military Prediction Scores for Massive Transfusion

The Larson Score

The Larson score was developed in a cohort of 1124 military combat patients, of whom 420 (37%) had an MT. [83] The score uses admission systolic blood pressure, heart rate, haemoglobin and base deficit to predict MT (Table 15.18). The chosen threshold of ≥2 points gave a sensitivity of 69% and specificity of 65%; however, it must be considered that this population has an unusually high proportion of patients who receive an MT. Among patients who scored zero points on the Larson score, 16% received MT. An evaluation of patients predicted not to require MT who did receive one suggested that severe thoracic and abdominal injuries were present, and thus the index of suspicion would remain higher for the clinician despite the negative score.

The Larson score was validated on a large cohort of the TR-DGU alongside other predictive models and performed with sensitivity 70.9%, specificity 80.4% and an AUROC of 0.823 [11]. This was the first validation of the score on a civilian database with a significantly lower proportion patients requiring MT (5.6%). It was validated again on a civilian population of 392 patients, with 9.2% receiving MT, and had a sensitivity of 76.5% and specificity of 77% [58]. This later validation used results available in the prehospital setting to expedite the provision of blood products. This demonstrated the ease and speed with which the score can be calculated, if the necessary blood tests can be taken before arrival or indeed early upon arrival.

Table 15.17 The Baker Model [82]

Variable	Value	Points
Systolic blood pressure	<90 mmHg	1
Heart rate	>120 bpm	1
Glasgow coma scale	<9	1
'High-risk injury'	Yes	1

The Schreiber Score

The Schreiber score was created at two combat support hospitals on military casualties. The development cohort comprised 558 patients, of whom 247 (44.3%) received MT. [84] Three variables were identified as increasing the odds ratio for MT: haemoglobin ≤11 g/dL, INR > 1.5, and penetrating mechanism (Table 15.18). The AUROC for these variables was 0.80 on the development cohort.

The Schreiber score was validated on a civilian cohort from the TR-DGU with a rate of MT of 5.6% [11]. The threshold chosen in this validation was ≥1 point because it produced the most favourable combination of sensitivity and speci-

Table 15.18 Military Massive Transfusion Prediction Scores [83–85]

The Larson Score		The Schreiber Score		The McLaughlin Score	
Variable	Value	Variable	Value	Variable	Value
Haemoglobin	<11 g/dL	Haemoglobin	≤11 g/dL	Heart rate	>105 bpm
Base deficit	≤ − 6 mmol/dL	INR	>1.5	SBP	<110 mmHg
Heart rate	>110 bpm	Penetrating mechanism	Yes	pH	<7.25
SBP	<110 mmHg			Haematocrit	<32%

SBP, systolic blood pressure; *INR*, international normalised ratio

ficity: 85.8% and 61.7%, respectively. The AUROC was 0.8.

The prominence of penetrating mechanism in this prediction model makes it difficult to use in the civilian trauma population, the vast majority of whom sustain blunt trauma. Of the patients in the TR-DGU validation, only 5% had a penetrating mechanism, so very few cases would have scored three points. The presence of haemoglobin and INR is also a limitation: while haemoglobin can be very quickly measured, INR takes 40–60 minute to return in a standard emergency department and would thus be considered too slow to be helpful in activating a massive transfusion protocol.

The McLaughlin Score

The McLaughlin score was developed at a single combat support hospital on 302 military casualties [85]. Of the included patients, 80 (26%) received MT. Multivariate regression analysis was performed on several potentially predictive factors and four were found to be significantly correlated with MT: heart rate, systolic blood pressure, haematocrit and pH (Table 15.18). A formula is applied to the values for each variable to produce a likelihood of requiring MT:

$$\log(\mathrm{pl}(1-p) = 1.576 + (0.825 * \mathrm{SBP}) + (0.826 * \mathrm{HR}) + (1.044 * \mathrm{Hct}) + (0.462 * \mathrm{pH})$$

The score was 59.4% sensitive and 77.4% specific on internal validation. When each variable was dichotomised, the likelihood of MT increased with the presence of increasing numbers of risk factors; however, even patients with zero of the criteria had an 11% chance of receiving MT.

An external validation of the McLaughlin score in a rural setting revealed a sensitivity 15.8% and specificity 98% [62]. The authors used a threshold of all four variables, which was associated with an 80% incidence of MT, as their cut-point. The cumbersome formula required to produce a likelihood of MT is a limitation only in as much as it must be used initially, when implementing this score into a new trauma centre, to decide what is the appropriate threshold to activate the MTP based on the characteristics of the unique patient population.

Conclusion

Early recognition of severely injured, bleeding trauma patients with complicating trauma-induced coagulopathy would allow timely, targeted treatment aimed at stopping the bleeding and correcting lethal coagulation defects in these vulnerable patients. Massive transfusion of blood products is a potentially life-saving treatment in extreme cases and corresponding protocols, including logistics, need to be timely activated. The prompt activation of a massive transfusion protocol is crucial to its success: delays in the provision of blood products may dramatically decrease survival rates. Furthermore, massive transfusion protocols encourage the provision of blood products in ratios that closely resemble the composition of whole blood. Identification of patients who will receive a massive transfusion allows treating teams to aim for an ideal ratio. Patients can be identified as requiring massive transfusion using laboratory or point-of-care tests or through the use of prediction tools. The scores described and all summarized in Table 15.19 aim to predict the requirement of massive transfusion through physiological, biochemical or radiological signs. While these scores have been thoroughly and carefully developed, none have been proven to be consistently or reliably accurate. Importantly, no large prospective trials have investigated massive transfusion prediction scores including validation. These scores are limited by their retrospective validations, reliance on slow laboratory tests, cumbersome formulae and low sensitivity and specificity. The balance between sensitivity and specificity has been difficult to assess: a high sensitivity is important because the harms of delaying blood transfusion due to a patient not being identified as needing one are great; however, a low specificity inflicts

Table 15.19 Overview and Comparison of massive transfusion prediction scores

	Number variables	Laboratory tests	Imaging	Prehospital calculation	Sensitivity range	Specificity range
ABC	4	No	Yes	No	47–90%	34–86%
TASH	7	Yes	Yes	No	56–84.4%	50–80%
PWH	7	Yes	Yes	No	3.4–92.9%	59.8–99.1%
Vandromme	5	Yes	No	No	53.4–78.9%	76.2–97.6%
SI	2	No	No	Yes	54.2–61.5%	72.3–93.1%
ETS	6	No	Yes	No	84.2–95%	60.8–92.5%
MTS	7	Yes	Yes	No	85%	41%
TBSS	5	Yes	Yes	No	97.4%	96.2%
Code red	3	No	No	Yes	89–91%	–
DMBT	7	Yes	Yes	No	78.2%	89.2%
RABT	4	No	Yes	No	84%	77%
Baker	4	No	No	Yes	100%	98%
Larson	4	Yes	No	No	70.9%	80.4%
Schreiber	3	Yes	No	No	85.8%	61.7%
McLaughlin	4	Yes	No	No	59.4%	77.4%

Number variables, number of variables required to calculate score; laboratory tests and imaging, whether these modalities are components of the prediction score; prehospital calculation, whether the score can be calculated before arrival at the emergency department; sensitivity and specificity range, range of results from published development and validation studies

the risk of potentially wasting valuable blood products. While there is no substitute for a clinician's *gestalt*, an effective massive transfusion trigger will increase efficiency and promote consistently excellent care in the trauma environment. Further study is needed to identify scores which adequately predict coagulopathy and massive transfusion to reduce mortality from traumatic injuries and haemorrhage.

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16 Prothrombin and Partial Thromboplastin Time

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General Description and Clinical Use of Basic Coagulation Tests – PT and PTT

Basic plasma coagulation function is readily assessed with a few simple in vitro laboratory tests: prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), and quantitative fibrinogen determination. Traditionally, the PT and PTT assays were developed for diagnostic purposes unrelated to diagnosing or treating trauma patients. The PTT was originally used to identify subjects with hemophilia and subsequently to assay the level of antihemophilic factor VIII in hemophilia A patients [1] or factor IX in hemophilia B patients. The PT was initially used to assay the prothrombin level [2, 3] and its principal clinical utility now is in monitoring the adequacy of warfarin anticoagulation or providing an early diagnosis of patients with coagulopathy due to liver disease.

Figure 16.1 depicts the well-known "coagulation cascade" culminating eventually in thrombin generation. This model of the coagulation process was derived from the biochemical interactions of the coagulation proteins and describes very well the mechanisms of the PT and PTT assays.

While the PTT assesses the intrinsic limb of the coagulation system (prekallikrein, high-molecular-weight kininogen, factors XII, XI, IX, VIII), the PT assesses the extrinsic or tissue factor-dependent pathway that consists of tissue factor and factor VII. Both tests also evaluate the common coagulation pathway factors (prothrombin, V, X, and fibrinogen) involving all the reactions that occur after the activation of factor X (Fig. 16.1). Simultaneously drawn PT and PTT are needed to properly evaluate the coagulation system.

In PT, clotting is initiated by recalcifying citrated patient plasma in the presence of a thromboplastin (tissue factor) reagent. The PTT, on the other hand, is performed by recalcifying citrated plasma in the presence of a thromboplastic material that "does not" have tissue factor activity (hence the term partial thromboplastin) and a negatively charged substance (e.g., celite, kaolin, silica), which results in contact factor activation, thereby initiating coagulation via the intrinsic clotting pathway. Both PTT and activated partial

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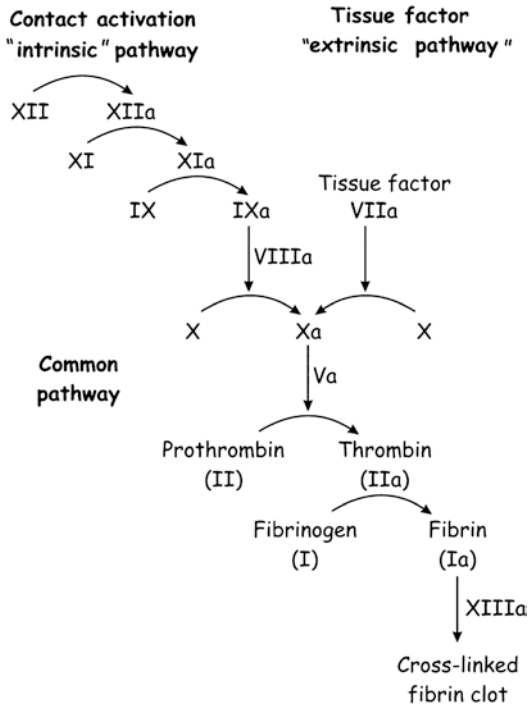


Fig. 16.1 The waterfall/coagulation cascade model of coagulation: the model is outlined as a Y-shaped scheme, with distinct "intrinsic" and "extrinsic" pathways initiated by FXII and FVIIa/tissue factor, respectively, with convergence of the two on a "common" pathway at the level of the FXa/FVa (prothrombinase) complex

thromboplastin time (aPTT) are used to test for the same functions; however, in aPTT, an activator is added that speeds up the clotting time and results in a narrower reference range. The aPTT is considered a more sensitive version of the PTT and is used to monitor the patient's response to heparin therapy. The reference range of the aPTT is 30–40 s. The reference range of the PTT is 60–70 s. The endpoint for the PT and PTT (and also thrombin time) is the time (in seconds) for the formation of a fibrin clot, which is detected by visual, optical, or electromechanical means.

A PT is expressed in seconds and/or as an international normalized ratio (INR). INR is the PT ratio that would result if the WHO reference thromboplastin had been used in performing the test. The PT/INR is most commonly used to measure the effect of warfarin and regulate its dosing as warfarin blocks the effect of vitamin K on hepatic production of extrinsic pathway factors. Utilizing the INR system of notation rather than the PT in seconds accounts for the variability of laboratory reagents used throughout the world and allows the effectiveness and dosing of warfarin therapy to be assessed by different laboratories with results that are consistent for patient care.

Prolongation of PT occurs with vitamin K deficiency (nutritional/antibiotic related), liver disease, DIC, deficiency of factors VII, II, and X, fibrinogen, oral direct thrombin, or Xa inhibitors. Prolongation of the PTT can occur with a deficiency of, or an inhibitor to, any of the clotting factors except for factor VII. Medications that prolong the PTT include heparin, direct thrombin inhibitors, and direct factor Xa inhibitors. Notably, certain lupus anticoagulants, which are antibodies directed against plasma proteins bound to anionic phospholipids, cause PTT prolongation by interfering with the in vitro assembly of the prothrombinase complex. These in vitro events are paradoxically associated with an increased risk of venous and arterial thrombosis.

It is important to note that the cascade concept of coagulation as depicted above has often incorrectly been interpreted as an "actual in vivo model of coagulation" implying two parallel running systems. Although the cascade model remains useful to explain the mechanisms and utility of the PT and PTT, it does not explain the physiology of hemostasis or thrombosis very well. A "cell-based" model of hemostasis as proposed by Hoffman et al. recognizes the major contribution of platelets to the clotting process, is more physiological, and shows that the two pathways have different functions and are both needed for adequate hemostasis. For details, please refer to Dr. Hoffman's description of the cell-based model of hemostasis [4, 5] in Chap. 3.

It is clear now that PTT and PT alone do not reflect the underlying physiology very well and furthermore they do not predict hemorrhagic or thrombotic predisposition. The absolute prolongation of the PT/PTT is not always clinically relevant. If the PT or PTT is abnormal, we can only determine the patient's risk of bleeding if we know the reason that the test is abnormal [6].

These screening tests are most useful in determining the cause of bleeding in a patient with hemorrhage, guiding the selection of other testing to look for specific factor deficiencies, and in guiding which blood components to transfuse to bleeding patients [6]. For example, a patient with an elevated PTT due to FVIII deficiency (hemophilia A) has a high risk of bleeding compared to a patient with an elevated PTT due to FXII deficiency who has no increased risk of bleeding and may actually be at risk for thrombosis. Likewise, a patient with a prolonged PTT due to the presence of a lupus anticoagulant usually does not have an increased risk of bleeding and may actually be at risk for thrombosis instead.

Role of PT/PTT in Coagulation Assessment in Trauma-Induced Coagulopathy

PT/INR and PTT are the most readily obtainable coagulation tests and so they remain the current standard for establishing a diagnosis of coagulopathy. While elevated PT is an integral part of the definition of early trauma-induced coagulopathy (ETIC) itself, PT and PTT have been used largely as an early indication of severity in trauma.

Hess et al. reported high prevalence of "abnormal coagulation tests" in patients at the time of presentation to trauma centers, even in the absence of significant crystalloid resuscitation. In an analysis of records of all patients admitted to a large urban trauma center from 2000 to 2006, abnormal PT was increasingly frequent, with abnormal results occurring in 5–43% of all trauma service admissions as the injury severity scores (ISSs) increased from 5 to more than 45. Similar trends were noted for PTT [7].

MacLeod et al. analyzed a trauma registry database of 7638 patients investigating PT, PTT, platelet count, age, ISS, presence of head injury, admission vital signs, and base deficit as predictors of mortality [8]. This study reported that in univariate analysis, abnormal PT had an odds ratio (OR) of 3.6 (95% confidence interval [CI], 3.15–4.08; $p < 0.0001$) for death with and an OR of 7.81 (95% CI, 6.65–9.17; $p < 0.001$) for deaths when combined with an abnormal PTT. In a multivariable regression model, PT and PTT remained independent predictors of mortality, whereas platelet count did not. The study concluded that a prolonged PT independently predicted a 35% increase in the likelihood of mortality and a substantially greater risk of mortality with a prolonged PTT as well [8].

Maegele et al. reported data from a large German Trauma registry database. They defined coagulopathy upon ER admission as abnormal prothrombin time test and/or platelets <100,000/μl. They detected evidence of ETIC in more than one third of patients (34.2%) upon presentation to ER. They noticed an increasing incidence of coagulopathy with increasing amounts of intravenous fluids administered prior to coagulation assessments. Coagulopathy was observed in >40% of patients with >2000 ml, in >50% with >3000 ml, and in >70% with >4000 ml of fluids administered [9].

Corroborating the data from German registry, Shaz et al. characterized the role of hemodilution in ETIC. They asserted that ETIC is associated with decreased factor activities without significant differences in thrombin and fibrin generation, suggesting that despite these perturbations in the coagulation cascade, patients displayed a balanced hemostatic response to injury. The "lower factor activities" are likely secondary to hemodilution and coagulation factor depletion. Thus, decreasing the amount of crystalloid infused in the early phases following trauma and early administration of coagulation factors through plasma resuscitation may prevent the development of severe ATC [10]. The data support the evolving picture that more severe injury, as demonstrated by increased ISS with resultant hypotension, can result in increased amounts of crystalloid administration in transit to the emergency department. This subsequent hemodilution results in a lower hematocrit and lower coagulation factor activities, most importantly factor VII, leading to the prolonged PT, which defines ETIC. Recent clinical randomized controlled trials investigating the efficacy of early prehospital plasma transfusion compared with standard of

care in trauma patients at risk for hemorrhagic shock have produced conflicting results [11, 12]. Whereas the PAMPer trial demonstrated a 30-day mortality benefit (23% vs 33%; $p = 0.03$) in the plasma-treated group, the COMBAT trial showed no difference in 28-day mortality (15% vs 10%; n.s.). Neither study reported clinically relevant improvements in standard assays of coagulation or viscoelastic testing following early plasma transfusion.

Other groups have corroborated these findings using different trauma registries. In a study of 182 patients from sub-Saharan Africa, the prevalence of coagulopathy was 54% (98/182) and mortality was significantly higher in the ATC group (29 deaths compared to 9 deaths in the non-ATC group). Notably, this study reported PTT as a strong independent predictor of mortality. They reported a significant difference in probability of survival between patients with elevated PTT and those with normal PTT ($p = 0.001$). Most deaths related to elevated PTT occurred early in the hospital stay, with the probability of survival becoming parallel in the two groups as time went on [13]. Hess et al. have also reported abnormal PTT to be associated with excess mortality with PTT values that were abnormal but below the conventional PTT transfusion triggers [7].

Isolated traumatic brain injury (TBI)-associated coagulopathy defined as PTT >40 s and/or a PT/INR >1.2 and/or a platelet count <120,000/μl has also been shown to be strongly associated with an unfavorable outcome [14].

Utility and Limitations of PT/PTT Measurements

While there are considerable benefits to PT/PTT assessments in trauma patients, there are also major limitations. While a prolonged PT remains a strong predictor of mortality and correlates with injury severity, it may not fully explain the mechanism of coagulopathy. It is very important to remember that these tests were originally designed to monitor oral anticoagulant therapy or diagnose hemophilia. The current testing procedures are performed on platelet-poor plasma at 37 °C and usually require 30–60 min to process by conventional methods [15], which may not be very helpful or timely in the management of acute trauma. Thus, for hemorrhaging trauma patients requiring acute interventions, these results may not be a contemporary and accurate reflection of coagulation function [5].

Nevertheless, currently available PT and PTT remain the most widely utilized and most cost-efficient first-line tests available for coagulopathy evaluation at most testing facilities especially so in resource-limited settings and smaller emergency room settings that are independent of major trauma centers [13]. They may also serve as an early warning about the severity of injury in a patient who presents with elevated PT but who is not actively bleeding. PT/INR point-of-care testing on arterial or venous whole blood has shown potential to circumvent the delays associated with conventional PT assays while maintaining the ability to detect ETIC during the initial assessment of trauma patients [16]; however, point-of-care coagulometers and laboratory-based PT/INR assays may show significant variability in this setting [17, 18].

Alternative Tests for Coagulopathy Evaluation in ETIC

Thromboelastography (TEG, Haemonetics, Braintree, MA) or rotational thromboelastometry (ROTEM, TEM International, GmbH, Munich, Germany) tests measure viscoelastic properties of the clot and have gained traction as testing methods that can be performed on whole blood. Viscoelastic testing is increasingly performed as "point-of-care" assays in the emergency department or perioperative settings providing quick turnaround times compared to testing performed in a centralized clinical laboratory [19–21].

A systematic review comparing the diagnostic accuracy of viscoelastic testing with the standard PT/INR for the diagnosis of TIC in adult trauma patients with bleeding found limited evidence to support the accuracy of either TEG or ROTEM and highlighted the need for further prospective,

randomized studies [22]. Since then to date, a single, randomized controlled trial in 111 adult trauma patients comparing goal-directed massive transfusion with either TEG or standard assays of coagulation (international normalized ratio, fibrinogen, platelet count) has been completed [23]. Compared with standard assays of coagulation, TEG-guided massive transfusion trauma resuscitation improved 28-day survival and resulted in fewer plasma and platelet transfusions in the early resuscitation period. Viscoelastic testing and applicability in ETIC will be addressed in more extensive detail in the ensuing chapters.

Conclusions/Summary

Current guidelines for the management of bleeding and coagulopathy in the adult trauma patient support early and repeated monitoring of coagulation with either standard coagulation assays (e.g., PT, INR, aPTT, platelet count, Clauss fibrinogen) or point-of-care assays (PT/INR) or viscoelastic testing [24].

PT/INR and PTT are the most readily obtainable coagulation tests and retain clinical utility to provide an early diagnosis of coagulopathy due to trauma. These assays have traditionally been used to detect and monitor TIC in hemorrhaging adult trauma patients. While both PT and PTT have been shown to be highly predictive of severity and mortality risk in trauma patients, they may not provide an accurate and contemporary description of the coagulation dysfunction in a trauma patient being resuscitated and may not be predictive of the bleeding or thrombosis risk per se. The results of PT/PTT testing typically have inadequate turnaround time to be useful to manage concurrent patients, but they retain some utility in assessing efficacy in treated patients as part of research protocols and ongoing databases.

Although the use of viscoelastic testing to monitor and guide transfusion in TIC continues to expand, there is currently limited evidence to support the superiority of viscoelastic testing compared with standard assays of coagulation for patient-centered outcomes [25]. Viscoelastic testing may provide more rapid and comprehensive assessments of the hemostatic system in trauma. As better testing algorithms for diagnosing and treating hemorrhage and/or thrombosis are developed, clinicians should suspect and empirically treat acute coagulopathy in patients at risk. It is likely that ongoing development and validation of robust point-of-care tests of coagulation and thrombosis will ultimately permit better tailoring of resuscitation to the individualized needs of each trauma patient.

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Fibrinogen Assays

17

Christoph J. Schlimp and Herbert Schöchl

General

Fibrinogen plays a crucial role in haemostasis. It is not only the precursor of fibrin but has also a high affinity to glycoprotein IIb/IIIa receptors, which are expressed on activated platelets. Thus, fibrinogen contributes to both platelet aggregation and the formation of a stable fibrin network. Importantly, fibrinogen seems to be the most vulnerable coagulation factor, reaching critically low levels earlier than any other coagulation protein in the course of severe bleeding [1–4]. A variety of reasons, such as major blood loss, dilution, consumption, hyperfibrinolysis, hypothermia and acidosis, are responsible for the rapid deterioration of the plasma fibrinogen concentration [5–7]. Importantly, low fibrinogen levels are strongly related to the amount of blood loss, the extent of shock and the severity of injury [8]. Recent guidelines recommend maintaining the plasma fibrinogen concentration above 1.5–2 g/L in bleeding trauma patients [9, 10]. Moreover, these guidelines strongly recommend early and repeated assessment of fibrinogen in trauma patients with substantial bleeding.

Fibrinogen, Principles of Measurement

More than 60 different methods for measuring plasma fibrinogen concentration have been described. The number depends on the type of activation method and read-out [11]. The four major fibrinogen assay groups are chronometric (Clauss), derived (prothrombin time), immunological and clottable. Typical read-outs are mechanical or optical. Moreover numerous reagents may multiply the possibilities of measurements [11].

Recently, in contrast to plasma fibrinogen measurements, whole blood fibrinogen measurement methods have been introduced, potentially providing faster results than plasma-based techniques [12–15].

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Chronometric Clauss Fibrinogen Measurement

Most commonly the Clauss method, which is a modification of thrombin time, is used to determine fibrinogen plasma concentration. As a note, consistency in the nomenclature of the Clauss method should be a goal as in the past it has been erroneously referred to as the "von Clauss" method [16]. In the original publication in German [17], "von" is not part of the name (i.e. a title of nobility), but simply means "written by" A. Clauss (Arnold Clauss). The Clauss method measures fibrinogen levels generated in a diluted, citrated platelet-poor plasma sample in the presence of an excess amount of thrombin (~100 U/mL). The time of the coagulation process is determined and extrapolated into a measurement of the functional fibrinogen concentration using calibration standards [18, 19]. The clotting time is inversely proportional to the amount of fibrinogen in the sample. Several methods have been developed to detect the fibrin clot endpoint generated by the Clauss assay. Automated coagulation analysers incorporating mechanical, electromechanical or photo-optical detection are commonly used. Mechanical devices detect the movement of a metal hook or the removal of a steel ball from a magnetic field upon clot formation. In contrast, electromechanical devices measure the effect of clot formation on the oscillation of a steel ball. The photo-optical techniques assess changes in light transmission [20]. Multiple commercially produced Clauss assays are available, with marked differences in thrombin concentration and source (e.g. bovine or human) and in the concentration of stabilisers, inhibitors and buffers [21]. In addition, a number of different reference plasmas are used as calibrators with substantial differences in composition between these products [20].

Derived Fibrinogen Measurement

Derived fibrinogen is based on the fact that the difference between baseline and maximum turbidity during prothrombin time (PT) measurement is proportional to the fibrinogen concentration between 0.5 and 16 g/L [22]. PT-derived fibrinogen measurement is established in some countries such as the UK, but it is not recommended by guidelines on fibrinogen assays because of the potential for discrepancies versus the Clauss method [21].

Whole Blood Fibrinogen Measurements

A new concept, which avoids prior centrifugation of the blood sample, is fibrinogen measurement in whole blood. Amukele et al. used an electromechanical device for the determination of whole blood fibrinogen, with acceptable results for the lower fibrinogen range but higher variations in fibrinogen levels above 4 g/L [12].

Ogawa et al. reported the "dry reagent method" (dry haematology), which assesses thrombin-induced clot formation in an oscillating magnetic field [14]. This technique has been introduced for whole blood PT, activated partial thromboplastin time and activated clotting time measurements. After adjusting for haematocrit an excellent correlation between whole blood fibrinogen measurements and the Clauss method has been observed.

This finding is in alliance with recent studies by Schlimp et al. in which fibrinogen concentration was measured in whole blood using a standard Clauss assay and a steel ball coagulometer [13]. The feasibility of this approach was tested with a Clauss assay using multiple porcine fresh blood samples obtained during in vivo bleeding, haemodilution and after treatment with haemostatic therapy. Two different Clauss assays were then tested using multiple human volunteers' blood samples diluted in vitro and supplemented with fibrinogen concentrate. After adjustment of the measured whole blood fibrinogen concentration with a quickly measureable haemoglobin-derived haematocrit, regression and Bland-Altman analyses of derived "plasma-equivalent" fibrinogen and measured plasma fibrinogen concentration were excellent in porcine and human blood samples, especially in the

ranges relevant to traumatic or perioperative bleeding. Hayakawa et al. also just reported whole blood coagulation analyser-measured fibrinogen levels within 2 min in critical care settings [15].

Fast whole blood fibrinogen measurements could be considered as an alternative to plasma fibrinogen measurement for acute bleeding management in trauma and perioperative care settings. Further studies are needed to prove this concept and determine the turnaround times for its clinical application in emergency rooms and operating theatres.

Immunologic and Clottable Fibrinogen Measurement

Immunologic and clottable fibrinogen measurement are frequently applied for research purposes but hardly used in clinical routine. Compared to the Clauss method or PT-derived measurements, immunologic assays are expensive and/or inappropriate in terms of turnaround times for clinical routine.

Moreover, immunologic assays count the number of molecules to some extent independent of its functionality. Therefore in trauma and shock it must be taken into account that an immunological assay shows total fibrinogen but not the actual functional fibrinogen concentration. However, from an experimental point of view the difference between an immunological and functional assay could give a hint to fibrinogen degradation occurring due to shock [23].

Plasma Fibrinogen, Shortcomings

Unfortunately, the Clauss method is not standardised. Numerous variations in respect to the read-out method (photo-optical, mechanical or electromechanical), type of calibrators, analyser platforms and assay brands could deliver substantially different results for the same plasma sample. A variety of studies showed that the measurement of plasma fibrinogen concentration can be affected by the type of device [24, 25], reagents [25], assay methods [26] and calibrators [27, 28] used. It has been shown recently that significant differences exist in the performance of assays for measuring plasma fibrinogen concentration, particularly between different laboratories ([16] and Fig. 17.1).

All of these variations have the potential to influence the result, especially in bleeding patients where further clinical variables (e.g. haemodilution, hydroxyethyl starch or the presence

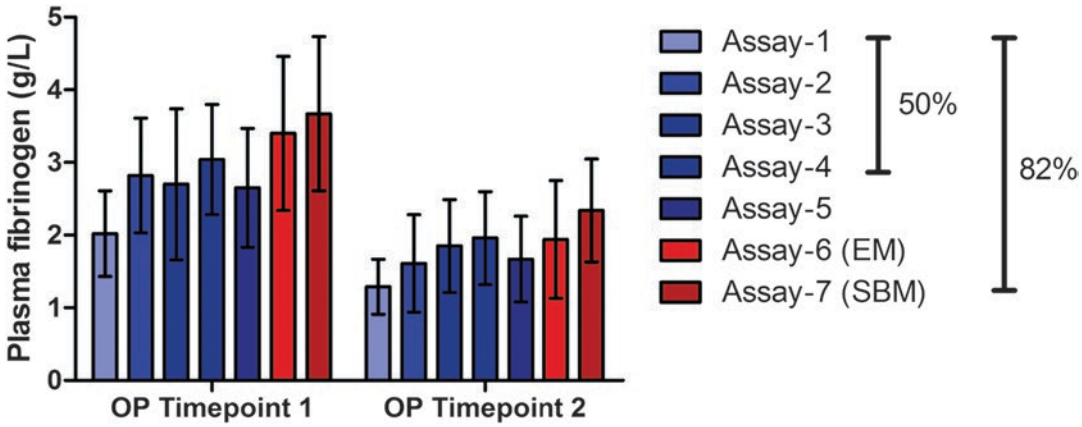


Fig. 17.1 Mean (standard deviation) values of fibrinogen concentration measurements obtained from the same set of plasma samples measure with seven different Clauss assays. Assays 1–5 represent assays with photo-optical read-out, assay 6 with an electromechanical (EM) read-out and assay 7 with a steel ball mechanical (SBM) read-out. Differences in the absolute fibrinogen concentration up to 82% have been observed [16]

of fibrin/fibrinogen degradation products or lipids) may amplify any differences between measurement methods. It has been reported that the presence of artificial colloids (e.g. dextran or hydroxyethyl starch) significantly raises measured fibrinogen concentration above that predicted by the dilutional effect [29]. Thus, when high volumes of synthetic colloids are infused in the course of massive transfusion, hypofibrinogenaemia may potentially be overlooked. Adam et al. reported that photo-optical methods significantly overestimate the fibrinogen concentration in blood diluted with hydroxyethyl starch [22, 30]. Fibrinogen concentration was overrated by >80% and >110% with 30% and 50% dilution, respectively. Similar observations have been made by Fenger-Eriksen et al. [24].

The most important shortcoming of plasma-based fibrinogen measurements is the unacceptable long turnaround time. Davenport and co-workers reported a median turnaround time for standard coagulation assessment of 78 min with an interquartile range of 62–103 min [31]. This is in line with a French survey, which reported a corresponding median time of 88 min with a range of 29–295 min [32]. This significantly limits the clinical value of PT-derived and Clauss measurements of fibrinogen. A much reduced turnaround time (14 min) has been reported and was achieved by rapid centrifugation, swift sample transport, optimal communications and an extended calibration range [33]. However, in most centres, it is doubtful whether this could be implemented in clinical routine.

Fibrin-Based Viscoelastic Tests

Modern viscoelastic methods such as thromboelastometry (ROTEM®, TEM International GmbH, Munich, Germany), viscoelastometry (ClotPro, enicor GmbH, Munich, Germany), thrombelastography/resonance method (TEG®, Haemonetics Corp., Braintree, MA, USA) or sonic estimation of elasticity via resonance sonorheometry (Quantra Hemostasis Analyzer, HemoSonics LLC, Charlottesville, VA) provide information on the speed of initiation of coagulation, the kinetics of clot growth, the clot strength and the potential breakdown of the clot . Specific assays, such as the ROTEM FIBTEM, ClotPro FIB test, TEG functional fibrinogen (FF) and Quantra fibrinogen contribution (FCS), are designed to assess the fibrin polymerisation in whole blood by assessing clot strength in the presence of a platelet inhibition [34–37]. It allows specific evaluation of the fibrin component of the clot (Fig. 17.2). Fibrin clot strength is primarily (though not exclusively) dependent on fibrinogen and activated FXIII. Thus, any functional impair-

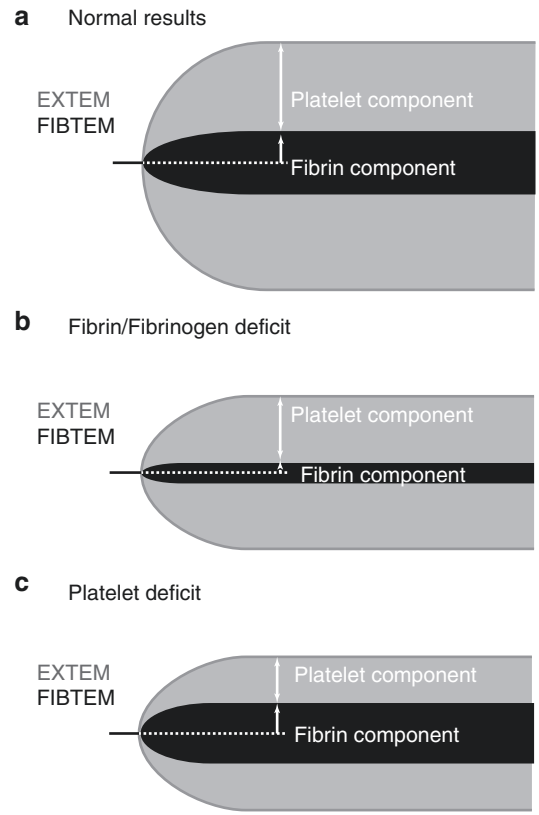


Fig. 17.2 EXTEM (extrinsically activated test) provides rapid information about the overall clot formation, whereas FIBTEM (extrinsically activated test plus cytochalasin D) focuses within the same time frame on the fibrin polymerisation of the clot after platelet inhibition (**a**). Fibrinogen and platelets both contribute to overall clot firmness. However, by only performing the EXTEM assay, fibrin(ogen) deficit (**b**) and platelet deficit (**c**) cannot be distinguished. With the supplementary platelet inhibition assay, the two coagulopathic conditions can be separated

ment of fibrinogen (e.g. by colloids) will diminish the entire clot strength [38].

Data suggest that differences between FF and FIBTEM test results may be encountered, and therefore ROTEM and TEG results must not be used interchangeably [35, 39–44]. A new assay (ROTEM assay: FIBTEM PLUS), which has been studied experimentally and clinically, provides higher effectiveness to inhibit the platelet contribution to clot strength (Fig. 17.3). Thus, a combination of cytochalasin D and a glycoprotein IIb/IIIa blocker allows a complete inhibition of platelet contribution and therefore more accurate information on the fibrin-based clot strength [39, 42]. The recently released ClotPro FIB-test already uses dual platelet inhibition with cytochalasin D and a synthetic glycoprotein IIb/IIIa antagonist [37].

Recent studies raised the question to which extent haematocrit influences whole blood viscoelastic test results [45–48]. It is important to keep in mind that the actual fibrin clot strength in whole blood only partially reflects plasma fibrinogen concentration, but gives a more comprehensive picture of fibrin polymerisation in whole blood. Moreover, it has been reported recently that fibrinogen interacts with erythrocytes [49, 50], and fibrinogen receptors on erythrocytes have been discovered [51, 52]. Binding of fibrinogen on these receptors might potentially interfere with clot strength. Although the amplitude of fibrin-based viscoelastic tests can be used to guide fibrinogen supplementation, we strongly discourage the reporting of functional fibrinogen in the way of a directly derived and calculated concentration (mg/dL) for the above-mentioned reasons [38]. However, when performed in platelet-free plasma an extrinsically activated thromboelastometric measurement correlates highly with the actual fibrinogen concentration [13].

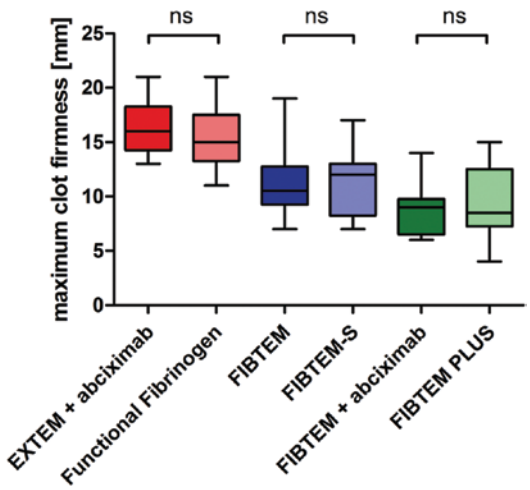


Fig. 17.3 Data from viscoelastic assays designed to assess the functional capacity of fibrinogen in whole blood. Graph shows the maximum clot firmness (median, interquartile range and range). All assays are activated extrinsically (tissue factor) and platelet function was inhibited either with a glycoprotein-IIb/IIIa receptor inhibitor (EXTEM + abciximab and functional fibrinogen), cytochalasin D (FIBTEM or FIBTEM-S) or a combination of both (FIBTEM + abciximab and FIBTEM PLUS). Unless stated "ns" (not significant), maximum clot firmness from all assays resulted in significant between-test differences [39]

Clinically, it has also been shown that the FIBTEM assay may have potential for early prediction of massive transfusion [53]. An important advantage of both the FIBTEM and FF assays is a short turnaround time. ROTEM and TEG analyses are performed in whole blood, thus avoiding the need for prior centrifugation of the blood sample. For ROTEM, a mean manipulation (set-up) time of 2 min 51 s has been reported for trained physicians [54]. Therefore, the first ROTEM test results allowing to estimate fibrin base clot strength, such as the clot amplitude at 5 min (A5), can be expected in around 8 min. Turnaround times for the FF assay have not been reported until now. Guidelines of the European Society of Anaesthesiology, and others, propose to use viscoelastic measurements in traumatic or perioperative bleeding patients [9, 10].

Plasma Fibrinogen Estimation by Surrogate Parameters

Rapid estimation of plasma fibrinogen in major trauma patients upon emergency room admission has been recently proposed, based on the results of blood gas analysis including the measurement of haemoglobin (Hb) and base excess (BE) as well as calculation of the Injury Severity Score

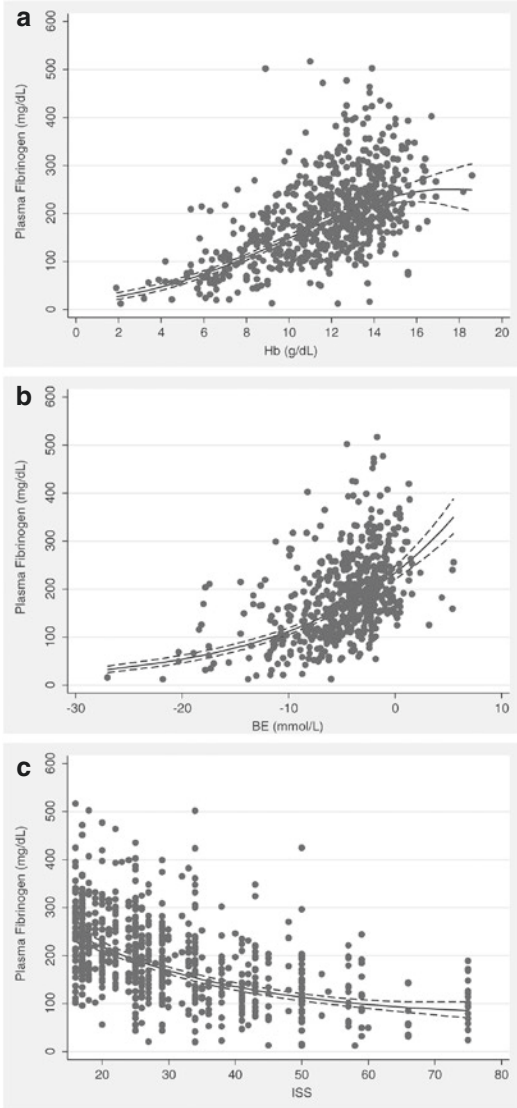


Fig. 17.4 Plasma fibrinogen concentration (mg/dL) versus (**a**) haemoglobin (Hb, g/dL), (**b**) base excess (BE, mmol/L) and (**c**) Injury Severity Score (ISS). The fitted regression line (solid) is shown along with a corresponding 95% confidence interval (*dotted lines*) [8]

[8] (Fig. 17.4). In a study of severely injured patients ($n = 675$) admitted with an Hb <12 g/dL, 74% had low (<2 g/L) and 54% had critical (<1.5 g/L) plasma fibrinogen levels. Of patients admitted with Hb <10 g/dL, 89% had low and 73% had critical plasma fibrinogen levels. These values increased to 93% and 89%, respectively, among patients with an admission Hb <8 g/dL. Sixty-six per cent of patients with only a weakly negative BE (<−2 mmol/L) showed low plasma fibrinogen levels. Of patients with BE <−6 mmol/L upon admission, 81% had low and 63% had critical plasma fibrinogen levels. The corresponding values for BE <−10 mmol/L were 89% and 78%, respectively (Table 17.1).

Table 17.1 Percentage of patients with adequate, low and critical plasma fibrinogen levels (FIB) when combining the parameters of Hb (g/dL) and BE (mmol/L) of severe trauma patients on admission [8]

Adequate FIB (≥200 mg/dL)	Hb ≥12.0	Hb 11.9–10.0	Hb 9.9–8.0	Hb <8.0
BE ≥ −2	63%	73%	25%	50%
BE −2.1 to −6	57%	30%	24%	17%
BE −6.1 to −10	50%	21%	5%	3%
BE < −10	42%	0%	0%	4%
Low FIB (<200 mg/dL)	Hb ≥12.0	Hb 11.9–10.0	Hb 9.9–8.0	Hb <8.0
BE ≥ −2	37%	27%	75%	50
BE −2.1 to −6	43%	70%	76%	83%
BE −6.1 to −10	50%	79%	95%	97%
BE < −10	58%	100%	100%	96%
Critical FIB (<150 mg/dL)	Hb ≥12.0	Hb 11.9–10.0	Hb 9.9–8.0	Hb <8.0
BE ≥ −2	6%	11%	8%	50%
BE −2.1 to −6	18%	36%	56%	75%
BE −6.1 to −10	11%	68%	65%	93%
BE < −10	33%	67%	92%	93%

Conclusion

Many different plasma fibrinogen measurement methods are available. However, most commonly the Clauss method is used in order to measure fibrinogen plasma concentration. Several factors, including type of device and reagent, have been shown to affect the results of fibrinogen concentration measurements by the Clauss method and clinically significant differences exist between the performances of assays. Turnaround times for plasma fibrinogen in the clinical settings of a bleeding patient may be considered too long. Measurement of whole blood fibrinogen in perioperative or traumatic bleeding may be a future option to rapidly determine fibrinogen concentrations and can be easily calculated for plasma values as well.

Plasma fibrinogen estimation in trauma patients on admission by surrogate parameters has been proposed. Viscoelastic tests estimating fibrin-based clot strength in whole blood are increasingly used to faster estimate the need for fibrinogen supplementation in the bleeding trauma patient.

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Rotational Thromboelastometry (ROTEM®)

18

Klaus Görlinger, Daniel Dirkmann, and Alexander A. Hanke

Dedication This chapter is dedicated to my wife Dr. Anke Görlinger, who passed away much too early on December 27, 2019. Without her support, this work could not have been done.

Thromboelastometry Basics

The ROTEM® Device

Rotational thromboelastometry (ROTEM®, TEM Innovations GmbH, Munich, Germany and Instrumentation Laboratory, Bedford, MA, USA) is a whole blood viscoelastic hemostasis analyzer, which evolved from the original thromboelastography (TEG) system, introduced by Helmut Hartert in 1948 and in the 1990s by Andreas Calatzis to the ROTEG® and later ROTEM® *delta* system [1, 2]. Although the TEG® (5000) and ROTEM® (*delta*) devices still share some similarities, there are several distinct differences with regard to measurement technique, assays, and measurement variables (Fig. 18.1) [3, 4].

The semiautomated ROTEM® *delta* device consists of a compact measurement unit with four temperature-adjusted independent measurement channels, a pre-warming plate, a reagent tray, and an integrated personal computer, allowing for remote viewing and LIS (laboratory information system) connection. An attached touchscreen and a software-assisted automatic pipette are used to control the device and the specific ROTEM® software. This makes the device very user-friendly and reduces intra- and inter-operator variability of test results [5–7]. This allows for using the device in a multiuser environment, for example, in the emergency room, operating room, or at the intensive care unit. Furthermore, the user is guided through the measurement process by the ROTEM® device with instructions and pictograms, displayed on the touchscreen, and a help menu can be activated if support in result interpretation is desired. Of course, this does not substitute for adequate education in hemostasis and decision-making by the attending physician.

The new ROTEM® *sigma* device, CE marked in Europe since August 2015 and FDA-approval study actually running (2019), is still using the

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Fig. 18.1 (**a**) ROTEM® *delta* device (thromboelastometry) plus ROTEM® *platelet* module (whole blood impedance aggregometry), (**b**) ROTEM® *sigma* device and (**c**). ROTEM® *sigma* cartridge. (Courtesy of Klaus Görlinger, Tem Innovations)

proven viscoelastic pin-and-cup technology but avoids any pipetting as a cartridge-based, fully automated ROTEM® device [8–10]. The ROTEM® *sigma* device is designed for the use at the point of care, reduces the hands-on time to about 2 min, and eliminates any potential pipetting error and other user errors. ROTEM® *sigma* cartridges provide a panel of four ROTEM® assays. Actually, there are two different cartridges available: type 1 (complete) contains EXTEM, FIBTEM, INTEM, and APTEM for cases where the effect of an antifibrinolytic drug should be assessed; type 2 (complete + hep) contains EXTEM, FIBTEM, INTEM, and HEPTEM for cases where a differentiation between coagulation factor deficiencies and heparin or heparin-like effects might be necessary (see section "ROTEM® Assays"). Besides cardiovascular surgery and liver transplantation, this may apply for any cases with severe shock and endothelial/glycocalyx damage such as in severe trauma and postpartum hemorrhage [11–13]. Cartridge-based assays are labeled by the suffix C (e.g., EXTEM C). This allows for identifying the device type used to generate the data stored in the database.

Measurement Technique

The four viscoelastic measurement channels of the ROTEM® devices allow for using a panel of specific assays. This improves the diagnostic performance of the device compared to a mono-assay system activated by kaolin [14]. Accordingly, the ROTEM® device is not only suitable to detect a coagulopathy, timely, but also to differentiate between different coagulopathies, for example, between hypofibrinogenemia and thrombocytopenia, and is designed to guide hemostatic therapy in bleeding patients (Fig. 18.2). Each measurement channel in ROTEM® *delta* consists of a disposable cuvette fixed in a temperature-adjusted metal cup-holder and a disposable pin attached to a moving axis, stabilized by a ball bearing. In ROTEM® *sigma*, pins and cups, a metal mixing ball and lyophilized reagent beads are integrated into the ROTEM® *sigma* cartridges. The ROTEM® axis is alternatingly rotating forth and back by 4.75° twelve times per minute. After starting the test by recalcifying the citrated whole blood in the cup and adding an activator (tissue factor or ellagic acid), clot strands between pin and cup-wall are increasingly impairing the pin rotation. These changes in the pin movement are detected by a LED light-mirror-light detector system, and the consequential signal is processed and transformed by the computer into a thromboelastometric curve (temogram) finally (Fig. 18.3). In addition, specific ROTEM® parameters are calculated by the computer and displayed on the

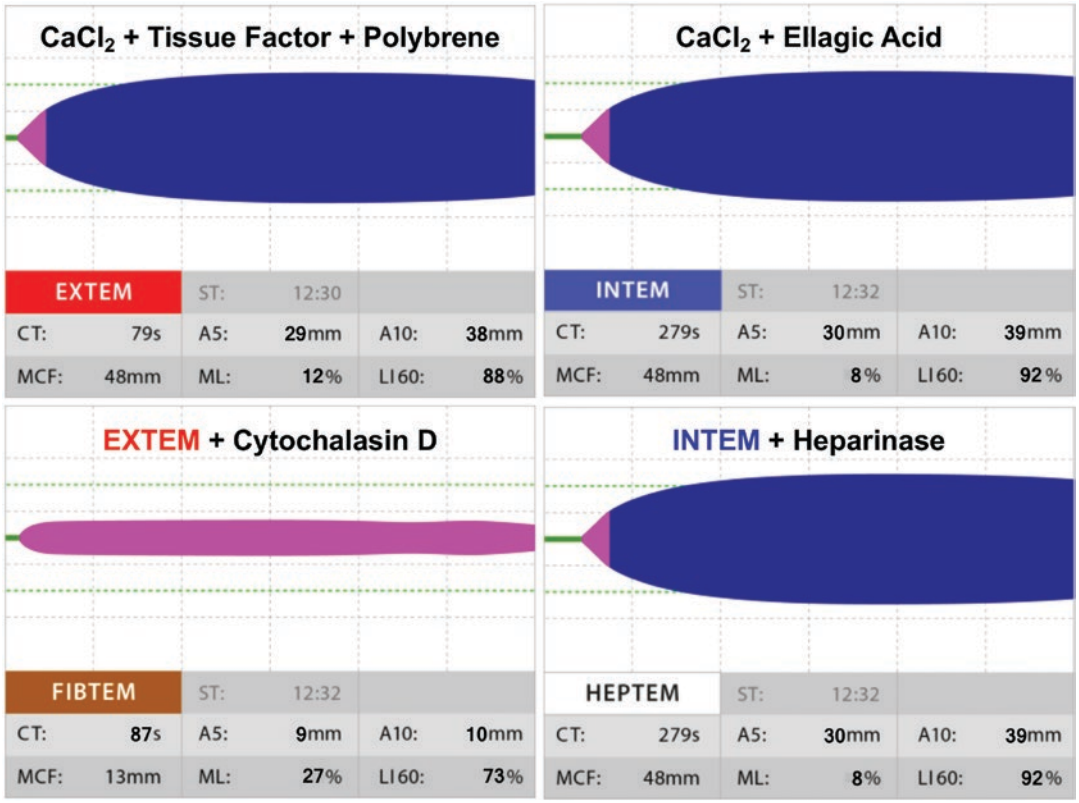


Fig. 18.2 ROTEM screenshot displaying four ROTEM® tests running simultaneously. EXTEM, FIBTEM (and APTEM, not displayed) contain polybrene and HEPTEM heparinase to eliminate a heparin effect. The test combination of EXTEM and FIBTEM allows for discrimination between thrombocytopenia and hypofibrinogenemia, and the test combination of INTEM and HEPTEM is used to detect a heparin effect. (Courtesy of Klaus Görlinger, Tem Innovations)

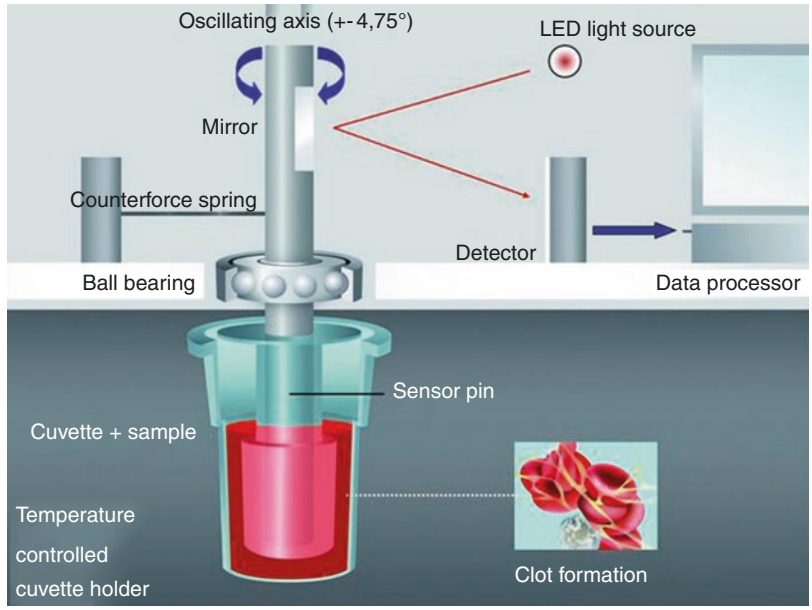


Fig. 18.3 Measuring principle of rotational thromboelastometry (ROTEM® *delta* and ROTEM® *sigma*). (Courtesy of Klaus Görlinger, Tem Innovations)

touch screen in real time. These technical modifications make the ROTEM® device on the one hand less susceptible to vibrations and movement artifacts, and on the other hand, allow for a continuous electronic quality control of the pin movement. Therefore, quality control using the reagents ROTROL® N and P is necessary once a week only, compared to daily QCs required for other viscoelastic test devices. This reduces costs and workload significantly. Furthermore, the device can be used in a mobile way at the point of care (e.g., in the emergency room, the operating room, the intensive care unit, or a satellite lab) and can even be moved around with the patient on a customized trolley providing uninterrupted power supply (Table 18.1). Accordingly, ROTEM® devices have successfully been used in military settings and other outdoor environments (e.g., mountaineering in the Himalaya and the Andes) [15–20].

ROTEM® Assays

Thromboelastometric assays use citrated whole blood (300 µL per assay), which is recalcified and activated by tissue factor (extrinsic pathway), ellagic acid (intrinsic pathway), or ecarin (direct prothrombin activation). Some assays contain further additives (Table 18.2). In contrast to the TEG system, all pipetting steps in ROTEM® *delta* are guided by the ROTEM® software and performed using an automated pipette. In ROTEM® *sigma*, the measuring process is fully automated. This allows for improved multiuser handling with lower intra- and inter-operator variability of the results when compared to other devices [5–7]. Besides the standard liquid reagents, lyophilized single-potion or single-use reagents (SUR) are available in Europe [21]. Since SURs contain all reagents needed for one assay, lyophilized in one vial, pipetting is minimized to adding 300 µL of citrated whole blood to the reagent vial and transferring the activated blood 5 s later to the ROTEM® cup. SURs are labeled by the suffix S (e.g., EXTEM S). Notably, extrinsically activated SURs do not contain a heparin inhibitor and, therefore, must not be used in patients with therapeutic anticoagulation with unfractionated heparin, for example, in cardiovascular surgery, or in any patients who might present an endogenous heparin-like effect due to

Table 18.1 Technical improvement in thromboelastometry and its impact on device performance [3]

Technical improvements in thromboelastometry (ROTEM®)	Device performance
Axis stabilized by a ball bearing	Low sensitivity to agitation and movement artifacts enables mobile use (even in military settings)
Contactless detection of pin movements by a LED light-mirror-light detector system	
ROTEM® trolley (with optional power supply)	
Software-assisted automatic pipette (ROTEM® *delta*) and full automatization (ROTEM® *sigma*) result in low intra- and inter-operator variability	User-friendly, higher precision and reproducibility of results; enables multiuser environment (bedside testing in the ER, OR, and ICU)
System-integrated personal computer and touchscreen	User-friendly
Test procedure guided by instructions and pictograms on the touchscreen	
Temogram overlays and help menu for support in result interpretation	
Continuous electronic quality control of pin movements (QC with control reagents only once a week necessary)	User-friendly; reduced workload and QC costs
Four channels for viscoelastic testing (ROTEM® *delta* and ROTEM® *sigma*)	Improved diagnostic performance; enables guided therapy with allogeneic blood products and coagulation factor concentrates ("theranostic approach"; "precision medicine")
Two channels for whole blood impedance aggregometry (ROTEM® *platelet* module)	
Panel of 8 ROTEM® *delta*, 5 ROTEM® *sigma* and 3 ROTEM® *platelet* assays	
Tissue factor activation (CT of 40–80 s compared to 4–8 min with kaolin activation)	Reduced turn-around-time (10–15 min) and short time-to-treat (in particular in combination with coagulation factor concentrates)
Early variables of clot firmness (A5, A10)	
Remote viewing and LIS (laboratory information system) connection	Real-time results at the bedside if the device is placed in the lab

Courtesy of Klaus Görlinger, Essen, Germany
ER emergency room, *ICU* intensive care unit, *OR* operating room, *QC* quality control

Table 18.2 ROTEM® *delta/sigma* and ROTEM® *platelet* assays

Assay	Activators and additives	Clinical comments
ROTEM ® delta/sigma assays		
EXTEM	$CaCl_2$ + recombinant tissue factor + polybrene	Deficiency of factors of the extrinsic pathway; VKAs (coumadin/warfarin) and DOACs; indication for PCC administration
FIBTEM	$CaCl_2$ + recombinant tissue factor + cytochalasin D + polybrene	Fibrin polymerization; dose calculation for fibrinogen concentrate or cryoprecipitate; hyperfibrinolysis; FXIII deficiency
APTEM	$CaCl_2$ + recombinant tissue factor + aprotinin/tranexamic acid + polybrene	Verifying the effect of antifibrinolytic drugs; differential diagnosis to platelet-mediated clot retraction and FXIII deficiency (in combination with EXTEM)
INTEM	$CaCl_2$ + ellagic acid	Deficiency of factors of the intrinsic pathway; unfractionated heparin (UFH) and protamine effects (in combination with HEPTEM)
HEPTEM	$CaCl_2$ + ellagic acid + heparinase	Testing in patients with very high heparin plasma concentrations; UFH and protamine effects (in combination with INTEM)

(continued)

Table 18.2 (continued)

Assay	Activators and additives	Clinical comments
NATEM NA-HEPTEM ECATEM	$CaCl_2$ $CaCl_2$ + heparinase $CaCl_2$ + ecarin	Tissue factor-expression on circulating cells (e.g., monocytes or malignant cells); other anticoagulants (e.g., LMWH in combination with NA-HEPTEM) Tissue factor-expression on circulating cells (e.g., monocytes or malignant cells) in blood samples with heparin or HLE; other anticoagulants (e.g., LMWH) (in combination with NATEM) Direct thrombin inhibitors (e.g., hirudin, argatroban, bivalirudin, dabigatran); not sensitive to heparin; new preparation (reagent beads technology) under development
ROTEM ® platelet assays		
ARATEM	Arachidonic acid (AA)	COX-1 (e.g., aspirin) and GPIIbIIIa receptor inhibitor effects; effects of CPB, extracorporeal assist devices, trauma and sepsis
ADPTEM	Adenosine di-phosphate (ADP)	ADP (P2Y12) (e.g., clopidogrel and prasugrel) and GPIIbIIIa receptor inhibitor effects; effects of CPB, extracorporeal assist devices, trauma and sepsis
TRAPTEM	Thrombin receptor-activating peptide-6 (TRAP-6)	Thrombin (PAR-1) (e.g., vorapaxar) and GPIIbIIIa receptor inhibitor effects; effects of CPB, extracorporeal assist devices, trauma, and sepsis

Courtesy of Klaus Görlinger, Essen, Germany
COX-1 cyclooxygenase-inhibitor 1, *CPB* cardiopulmonary bypass, *DOACs* direct oral anticoagulants, *HLE* heparin-like effect, *LMWH* low molecular weight heparin, *PAR-1* protease-activated receptor 1, *PCC* prothrombin complex concentrate, *VKA* vitamin K-antagonists

endothelial glycocalyx degradation, for example, in patients with cirrhosis or severe shock [11–13, 22]. The ROTEM® system provides various activated assays which in combination considerably improve the diagnostic performance of the device in comparison to a mono-assay system [14]. Here, extrinsically activated assays (EXTEM, FIBTEM, and APTEM), intrinsically activated assays (INTEM and HEPTEM), an ecarin-activated assay (ECATEM), and non-activated assays (NATEM and NA-HEPTEM) are available. Actually, a new ECATEM assay, based on beads reagent technology, is under development.

Similar to the prothrombin time, **EXTEM** assays are activated by recalcification (star-tem® reagent, containing 0.2 mol/L calcium chloride) and addition of tissue thromboplastin (r ex-tem® reagents, i.e., recombinant tissue factor and phospholipids). Accordingly, since coagulation is initiated through the extrinsic pathway, initial thrombin generation, and hence initial clotting, mainly depends on the activity of the coagulation factors VII, X, V, II, and fibrinogen in the EXTEM test.

The **FIBTEM** assay consists of a modified EXTEM assay with the addition of a potent platelet inhibitor (cytochalasin D), which blocks platelet activation, shape change, and expression and activation of glycoprotein IIbIIIa (fibrinogen) receptors [23]. Thereby, platelet contribution to clot formation and clot strength is eliminated in this assay [24]. Accordingly, clot strength in FIBTEM is based on fibrinogen concentration and fibrin polymerization, solely, whereas clot strength in EXTEM depends on platelet count, platelet function, fibrinogen concentration, and fibrin polymerization. Therefore, the combination of EXTEM and FIBTEM allows for discrimination between thrombocytopenia and hypofibrinogenemia. The difference in clot strength between EXTEM and FIBTEM allows for estimation of the platelet part of clot firmness (referred to as PLTEM by some authors) [25].

A third extrinsically activated assay—the **APTEM** test—includes an antifibrinolytic drug (in the past aprotinin and nowadays tranexamic

acid (t ap-tem®)) allowing for in vitro assessment of an antifibrinolytic therapy.

All extrinsically activated liquid assays contain polybrene, a heparin inhibitor which allows for the immediate elimination of heparin effects (up to 5 units unfractionated heparin per mL). This enables the use of these tests even in heparin-treated patients, for example, during cardiopulmonary bypass [26].

INTEM assays are activated by recalcification and addition of ellagic acid and phospholipids. Due to the intrinsic activation, similar to the activated partial thromboplastin time, initial thrombin generation, and clot formation in INTEM, mainly depends on coagulation factors XII, XI, IX, VIII, X, V, II, and fibrinogen. As in EXTEM, clot firmness reflects both platelet and fibrin contribution to the clot. In contrast to all extrinsically activated assays, INTEM does not contain a heparin inhibitor in order to detect the effect of unfractionated heparin. However, a modified INTEM assay, containing additional heparinase (**HEPTEM**), can be used in combination with INTEM in order to reveal (residual) heparinization or protamine overdose. Here, the INTEM/HEPTEM CT ratio correlates well ($r = 0.72$) with the anti-Xa-activity [11].

The **NATEM** assay is activated by recalcification (star-tem® reagent) only. Therefore, the test is very sensitive to any endogenous activator such as tissue factor-expression on circulating monocytes in infection, sepsis, cirrhosis, and in patients treated with extracorporeal assist devices [27–30]. Therefore, this assay may be helpful to detect a pathophysiological change from trauma-induced coagulopathy (TIC) to disseminated intravascular coagulopathy (DIC). The **NA-HEPTEM** assay contains in addition heparinase in order to confirm a heparin or LMWH effect or to eliminate the interference of a heparin-like or LMWH effect in the detection of tissue factor expressing circulating cells.

Finally, the **ECATEM** assay uses the viper venom ecarin as an activator. Ecarin directly converts prothrombin to meizothrombin, which has already a low level of thrombin activity. Crucially, meizothrombin is inhibited by hirudin and other direct thrombin inhibitors (such as hirudin, argatroban, bivalirudin, and dabigatran), but not by heparin [31–33]. Clotting time in ECATEM is also unaffected by other enzymatic coagulation factor deficiencies (except prothrombin deficiency), coumadin (warfarin), direct factor Xa inhibitors (such as rivaroxaban, apixaban, and edoxaban), and the presence of phospholipid-dependent anticoagulants (such as lupus anticoagulant) [34]. A new ECATEM assay, using the reagent beads technology, is under development, providing better reagent stability, reproducibility, and user-friendliness [35].

ROTEM® Parameters

The ROTEM® test results are characterized by several ROTEM® parameters. Besides the standard ROTEM® parameters several other parameters are used for research only (Fig. 18.4, Table 18.3, and ROTEM® *delta/sigma* manual) [36–40]. ROTEM® *delta* reference ranges for the standard parameters are presented in Table 18.4 [41]. ROTEM® **reference ranges** are age- and gender-dependent and can slightly vary from country to country (e.g., between Europe and the USA) and even from hospital to hospital, dependent on the reference range population (Table 18.4) [8]. Therefore, these ROTEM® reference ranges are for orientation only, and it is recommended to establish hospital-specific reference ranges. Here, the reference range population, age, gender, blood sampling vials and technique, sample transport, and other pre-analytic factors may affect the results. Notably, specific age-related reference ranges for **neonates**, **infants, children** and trimester-related reference ranges for **pregnant women** have been published, too [42–47].

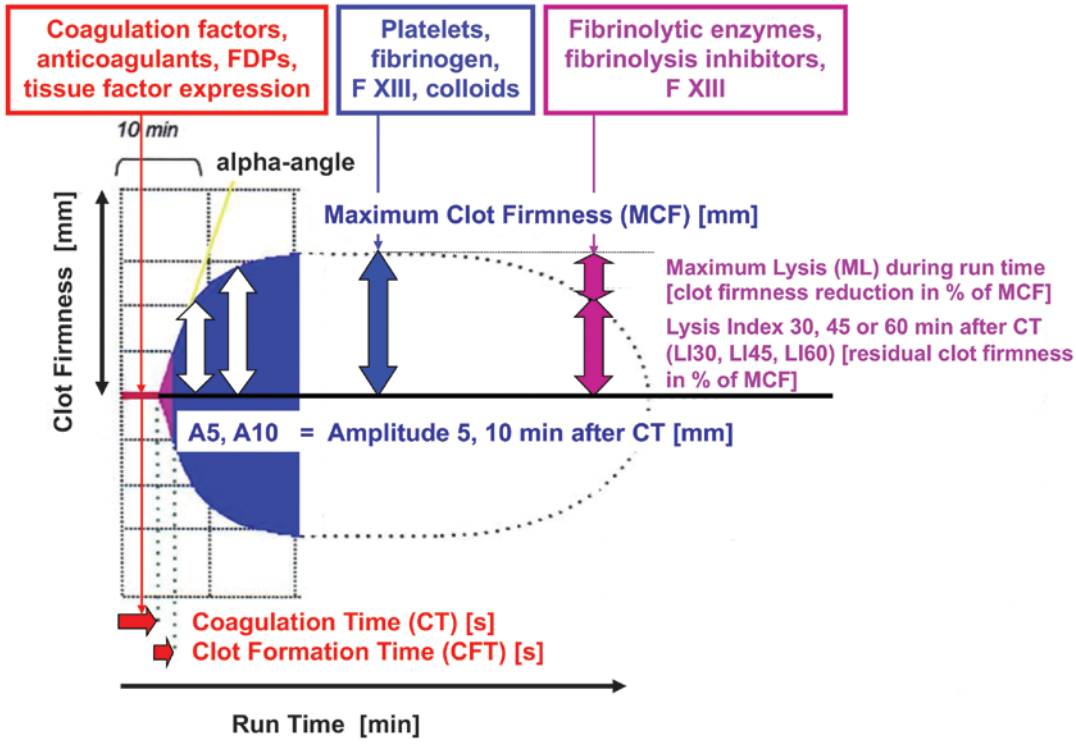


Fig. 18.4 ROTEM® trace ("temogram") displaying the clinically most important parameters and their informative value. FDPs = fibrin(ogen) split products. (Courtesy of Klaus Görlinger, Essen, Germany)

Table 18.3 ROTEM® *delta* parameters

Acronym	Parameter	Unit	Definition
Coagulation activation and clot polymerization parameters			
CT	Coagulation time	s	Time from test start until a clot firmness amplitude of 2 mm is reached
CFT	Clot formation time	s	Time between 2 and 20 mm clot firmness amplitude is achieved
α	Alpha-angle	degree (°)	Angle between the baseline and a tangent to the clotting curve through the 2 mm point
Clot firmness parameters			
A5	Amplitude at 5 min	mm	Amplitude of clot firmness 5 min after CT
A10	Amplitude at 10 min	mm	Amplitude of clot firmness 10 min after CT
A20	Amplitude at 20 min	mm	Amplitude of clot firmness 20 min after CT
MCF	Maximum clot firmness	mm	Maximum amplitude of clot firmness reached during the run time; *indicates that the final value is not reached, yet
Clot lysis parameters			
ML	Maximum lysis	%	Maximum lysis detected during the run time, described in % of MCF; * indicates that the final values is not reached, yet
LI30	Lysis index at 30 min	%	Residual clot firmness at 30 min after CT, described in % of MCF

Table 18.3 (continued)

Acronym	Parameter	Unit	Definition
LI60	Lysis index at 60 min	%	Residual clot firmness at 60 min after CT, described in % of MCF
LOT	Lysis onset time	s	Time from CT until clot firmness is decreased by 15% as compared to the MCF
Research parameters			
MCE	Maximum clot elasticity	–	MCE = 100 × MCF/(100−MCF)
G	Shear elastic modulus strength	–	G = 5000 × MCF/(100−MCF)
TPI	Thrombodynamic potential index	s^{-1}	TPI = MCE/CFT
LT	Lysis time	s	Time from CT until the clot firmness is decreased to 10% as compared to the MCF
CLR	Clot lysis rate	degree (°)	Angle between the baseline and the tangent to the declining clot firmness curve
Research parameters for the first derivative curve [36]			
maxV	Maximum velocity	mm/min	Maximum of the first derivative of the curve
maxV-t	Time to maximum velocity	s	Time from test start until the maximum of the first derivative of the curve is reached
AUC	Area under the curve	mm × min	Area under the curve of the first derivative from test start until MCF is reached

Courtesy of Klaus Görlinger, Tem Innovations

Table 18.4 ROTEM® *delta* reference ranges for adults (non-US)

ROTEM® *delta* reference ranges for adults (non-US) (reference ranges 2012-02 Ver0007; Tem Innovations GmbH; 2012-02-07)								
Assay	CT (s)	CFT (s)	α angle (°)	A10 (mm)	A20 (mm)	MCF (mm)	LI30 (%)	ML (%) within 1 h
EXTEM	38–79	34–159	63–83	43–65	50–71	50–72	94–100	<15
FIBTEM	–	–	–	7–23	8–24	9–25	–	–
APTEM	Comparison with EXTEM. A better clot formation in APTEM as compared to EXTEM demonstrate the in vitro effect of an antifibrinolytic drug (e.g., aprotinin or tranexamic acid)							
INTEM	100–240	30–110	70–83	44–66	50–71	50–72	94–100	<15
HEPTEM	Comparison with INTEM. A better clot formation in HEPTEM as compared to INTEM indicates the presence of heparin or heparin-like substances in the blood sample							
Disclaimer	ROTEM® *delta* reference ranges can slightly vary from country to country (e.g., between Europe and the USA) and even from hospital to hospital. They are dependent on the respective reference population, age, the blood sampling vials and technique, sample transport, and other pre-analytic factors. Therefore, these reference ranges are for orientation only, and it is recommended to establish hospital-specific reference ranges							

Courtesy of Klaus Görlinger, Tem Innovations

Coagulation Activation and Clot Polymerization Parameters

The thromboelastometric coagulation time (**CT**) in seconds corresponds to the reaction time (*r*) of TEG® assays. In ROTEM® assays, CT is defined as the time from the test start until a clot firmness amplitude of 2 mm is reached. In tissue factor activated tests, the CT is usually achieved within about 1 min. The CT reflects the speed of thrombin generation and is mainly affected by the enzymatic activity of coagulation factors (extrin-

sic or intrinsic, depending on the assay used), the concentration of anticoagulants and fibrin split products, as well as tissue factor expression on circulating cells (e.g., monocytes or malignant cells) [27–30]. Notably, CT in EXTEM, FIBTEM, and APTEM is often prolonged in blood samples with low fibrinogen. Therefore, CT prolongations have to be interpreted carefully if early FIBTEM clot firmness amplitude (A5 or A10) is low (see also Fig. 18.8a, b) [48].

The clot formation time (**CFT**) in seconds indicates the time between 2 and 20 mm clot firmness amplitude is achieved. The CFT corresponds to the kinetic time (k) of TEG® assays and reflects the kinetics of clot formation. CFT mainly depends on thrombin generation, platelet count, and platelet function, as well as fibrinogen concentration and fibrin polymerization.

The alpha angle (α) in degree (°) reflects the kinetics of clot formation, too, and is defined as the angle between the baseline and a tangent to the clotting curve through the 2 mm point.

Clot Firmness Parameters

One of the most important ROTEM® parameters is the maximum clot firmness (MCF) in millimeters which corresponds to the maximum amplitude (MA) of TEG® assays. MCF is defined as the maximum amplitude of clot firmness reached during the test. The clot amplitude reflects the mechanical strength of the clot and mainly depends on platelet count and platelet function, fibrin concentration, and fibrin polymerization, factor XIII activity, and colloids.

In order to speed up decision-making in severe bleeding, the amplitude of clot firmness 5 or 10 min after CT (**A5** or **A10**, respectively) is increasingly being used. Actually, A5 is not yet available in the USA. A20 is used during quality control measurements. A5 and A10 correlate very well with the MCF (Spearman's coefficient of 0.91–0.98) and allow for decision-making within 10–15 min after starting the test [6, 17, 25, 49–51]. EXTEM and INTEM A5 and A10 correlate with platelet count and fibrinogen concentration, FIBTEM A5 and A10 correlate well with plasma fibrinogen concentration, and PLTEM A5 and A10 (EXTEM A5 (A10)—FIBTEM A5 (A10)) correlates well with platelet count [25, 51–53]. Furthermore, low clot firmness values have been demonstrated to be associated with an increased incidence of hyperfibrinolysis. Here, an EXTEM A5 ≤ 35 mm can identify more than 90% of patients developing hyperfibrinolysis finally [54]. This is in line with the threshold of EXTEM A5 ≤ 35 mm reported by Davenport et al. to identify trauma-induced coagulopathy on arrival in the emergency room [55].

Clot Lysis Parameters

The clot lysis parameters maximum lysis (**ML**) and lysis indices 30 and 60 (**LI30** and **LI60**) provide information about the activity of fibrinolytic enzymes, fibrinolytic inhibitors, and factor XIII. ML detected during run is described as the difference between MCF and the lowest amplitude after MCF, displayed as % of MCF. LI30 and LI60 indicate the percentage of MCF still present 30 and 60 min after CT, respectively. In contrast, the TEG® lysis parameter LY30 and LY60 indicate the amount of lysis in the percentage of MA, 30 and 60 min after MA is achieved. The ROTEM® lysis onset time (**LOT**) in seconds is characterized by the time period from CT until 15% of clot lysis is achieved [56]. FIBTEM has been shown to be the ROTEM assay most sensitive and specific for fibrinolysis [57, 58].

ROTEM® Limitations

A major limitation of standard viscoelastic testing is its insensitivity to the effects of antiplatelet drugs (e.g., cyclooxygenase-1 (COX-1) inhibitors, and ADP ($P2Y_{12}$)-receptor inhibitors) [37, 38]. This limitation is caused by the generation of high amounts of thrombin in viscoelastic test systems, which mask the effects of antiplatelet drugs by stimulating the platelets via the thrombin-receptor pathway (protease-activated receptor (PAR) 1 and 4) [59]. Since thrombin is the strongest activator of platelets, the inhibition of other pathways (e.g., arachidonic acid- or ADP-pathway) does not affect viscoelastic test results in the presence of high amounts of thrombin.

ROTEM® *Platelet* Module

To overcome this limitation, **ROTEM®** ***delta*** can be combined with the **ROTEM®** ***platelet*** module, which is CE-marked in Europe since November 2013. It provides two channels for whole blood impedance aggregometry in addition to the four viscoelastic channels of ROTEM® *delta* (Figs. 18.1 and 18.5). Arachidonic acid (**ARATEM**), adenosine di-phosphate (**ADPTEM**), and thrombin receptor-activating peptide-6 (**TRAPTEM**) can be used as activators in ROTEM® *platelet*. The corresponding reagents are designed as user-friendly lyophilized single-use reagents.

Whole blood impedance aggregometry has been shown to detect the effect of COX-1 inhibitors and ADP-receptor inhibitors, effectively, and to predict stent thrombosis/ischemic events and bleeding/platelet transfusion in interventional cardiology and cardiac surgery, as well as mortality in severe trauma and sepsis [38, 60–76]. Furthermore, the effects of drugs, such as desmopressin and tranexamic acid, on platelet function can be monitored by whole blood impedance aggregometry [77–79]. However, it is not clear whether platelet transfusion is beneficial or even harmful in patients with early platelet dysfunction in severe trauma and sepsis [80–85].

Diagnosis of Trauma-Induced Coagulopathy

Davenport et al. demonstrated in 2011 that TIC is functionally characterized by a reduction in clot strength. Here, thromboelastometry could identify

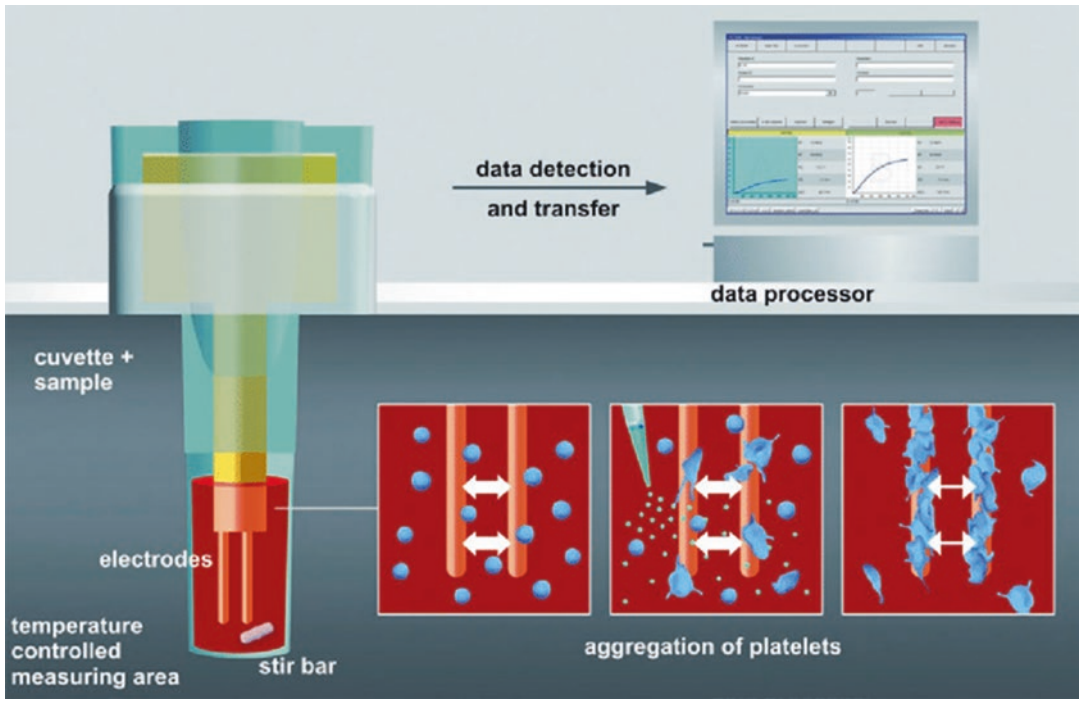


Fig. 18.5 Measuring principle of whole blood impedance aggregometry (ROTEM® *platelet*). Activated platelets are aggregating on the surface of the wires of the test cuvettes and, thereby, increasing the impedance between both wires. A magnetic stirrer prevents sedimentation of the blood cells during the 3 min incubation time. Arachidonic acid (ARATEM), adenosine diphosphate (ADPTEM), and thrombin receptor-activating peptide-6 (TRAPTEM) can be used as an activator. The measuring time is fixed to 6 min and the impedance curve is displayed on the screen. The main parameters are the area under the aggregation curve (AUC in Ω × min), amplitude at 6 min (A6 in Ω), and a maximum slope of the aggregation curve (MS in Ω/min). Citrate, heparin, or hirudin can be used as an anticoagulant in the blood sampling vial. Different waiting times and reference ranges have to be considered dependent on the anticoagulant used. (Courtesy of Klaus Görlinger, Tem Innovations)

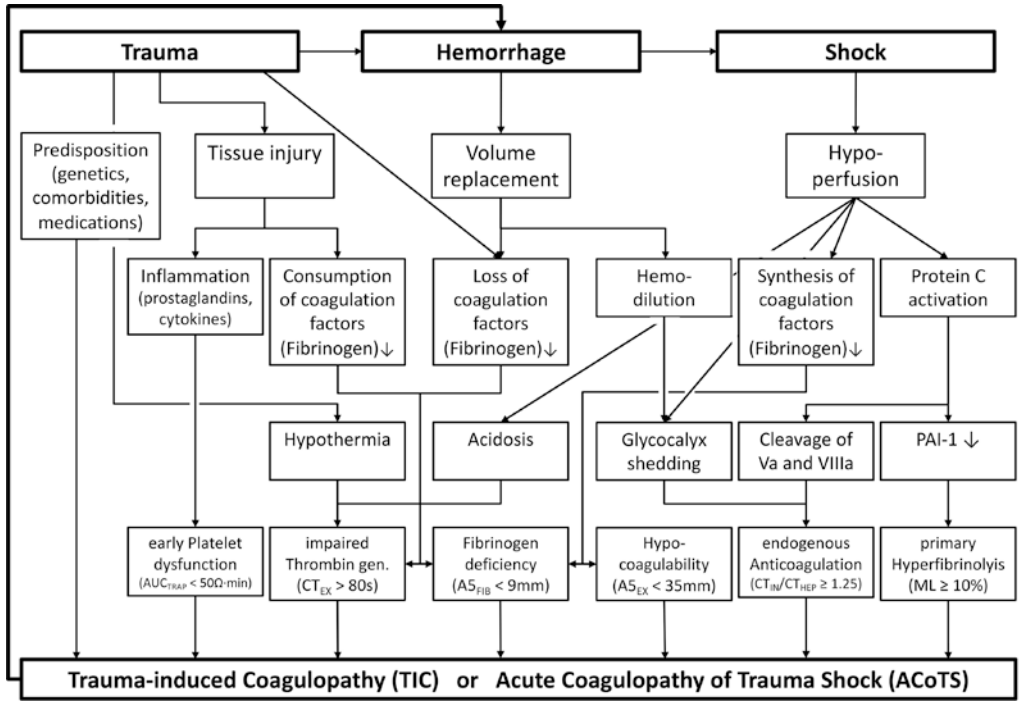


Fig. 18.6 Pathophysiology of trauma-induced coagulopathy (TIC) or acute coagulopathy of trauma and shock (ACoTS) and its detection by ROTEM® tests. $A5_{EX}$ amplitude 5 min after CT in EXTEM, $A5_{FIB}$ amplitude 5 min after CT in FIBTEM, AUC_{TRAP} area under the curve in TRAPTEM (whole blood impedance aggregometry, assessed by ROTEM® *platelet*), CT_{EX} coagulation time in EXTEM, CT_{HEP} coagulation time in HEPTEM, CT_{IN} coagulation time in INTEM, 5ML maximum lysis (within 1 h run time), PAI-1 plasminogen activator inhibitor-1. (Courtesy of Klaus Görlinger, Essen, Germany)

TIC with a threshold of EXTEM A5 ≤ 35 mm on arrival in the emergency room [55]. Besides EXTEM A5 (A10), other thromboelastometric and aggregometric parameters, such as FIBTEM A5 (A10), FIBTEM ML (LI60), EXTEM ML (LI60), EXTEM CT, INTEM/HEPTEM CT-ratio, and TRAPTEM AUC, can be helpful to characterize TIC and to guide hemostatic therapy (for details, see Fig. 18.6 and section "Thromboelastometry-Guided Hemostatic Therapy").

Prediction of (Massive) Transfusion

Since plasma transfusion may improve outcome only in patients requiring massive transfusion, whereas plasma transfusion in patients not requiring massive transfusion only shows an increased complication rate, early prediction of massive transfusion is crucial for decision-making to start plasma transfusion as a part of a massive hemorrhage protocol in severe trauma [86–91]. On the one hand, the need for massive transfusion can be predicted based on clinical scoring systems such as ABC and TASH score (ROC AUC, 0.779 and 0.890, respectively), and, on the other hand, based on thromboelastometric results (EXTEM or FIBTEM A5, A10, or MCF, or TRAPTEM AUC) on arrival in the emergency room [55, 76, 92–99].

In a retrospective analysis of 53 trauma patients, Leemann et al. reported that a decreased INTEM MCF < 50 mm (A10 < 44 mm) and a reduced hemoglobin level (≤10 g/dL) are independent risk factors for massive transfusion in severely injured trauma patients with an adjusted odds ratio (95% CI) of 8.47 (1.19–62.50) and 18.18 (2.73–125.00), respectively [95].

Davenport et al. found in a prospective, observational, single-center cohort study including 300 patients with full trauma team activation that a reduced EXTEM A5 (≤35 mm) is more accurate in predicting massive transfusion than INR (>1.2) [55].

In a retrospective study on 323 trauma patients published by Schöchl et al., both FIBTEM MCF and A10 were reasonable predictors of massive transfusion requirements. Here, patients admitted with a FIBTEM A10 ≤ 4 mm (MCF ≤ 7 mm), 84% received a massive transfusion (≥10 units of RBCs). None of the patients with an FIBTEM A10 ≥ 12 mm on admission received a massive transfusion finally [96].

These results have been confirmed by another prospective cohort study in 334 blunt trauma patients. Here, Tauber et al. observed that a reduced FIBTEM MCF (<7 mm) on admission to the trauma center strongly correlated with the need for red blood cell transfusion [97].

The high predictive value of early EXTEM and FIBTEM clot firmness parameter (A5 and A10) to predict massive transfusion (ROC AUC, 0.75–0.89) has been confirmed by two other large validation studies including overall 1954 trauma patients [98, 99].

Only trauma-induced platelet dysfunction in blood samples collected at the scene of injury or upon hospital arrival, characterized by an aggregation AUC in ROTEM® *platelet* TRAPTEM <53 Ω·min or ADPTEM <43 Ω·min, was superior in predicting massive transfusion or death within 6 h after injury with a ROC AUC of 0.97 and 0.95, respectively [76].

Prediction of Thrombotic/Thromboembolic Events

Three important mechanisms are involved in the pathophysiology of disseminated intravascular coagulation (DIC), microvascular thrombosis, and multiple organ failure: hypercoagulability, characterized by an increased clot firmness in EXTEM and INTEM, tissue factor (TF)-expression on circulating monocytes, characterized by a shorting of CT in NA-HEPTEM despite prolonged PT and aPTT, and hypo fibrinolysis/fibrinolysis shutdown, characterized by less than 3% lysis within 1 h [27–29, 100]. This triad results in delocalization/dissemination of clot formation and microthrombosis and a simultaneous shutdown of the physiologic fibrinolytic cleaning system. Accordingly, it seems to be important to detect the time point when TIC shifts to DIC in trauma patients [101–103]. This may also be one reason why tranexamic acid increased mortality in the CRASH-2 study when given later than 3 h after injury [104–106].

Clot Firmness in EXTEM, INTEM, and FIBTEM

In a prospective observational study in 69 patients with cardiovascular diseases, Dimitrova-Karamfilova et al. assessed the ability of routine coagulation tests (PT, aPTT, fibrinogen, and platelet count) and ROTEM® tests to identify patients with hypercoagulability and thrombotic complications. No statistically significant difference could be found for routine coagulation tests. In contrast, a significant difference in ROTEM® parameters could be observed in the 35 patients with thrombotic complications compared to the 34 healthy controls. In particular, EXTEM and INTEM CFT and MCF were able to identify patients with thrombotic complications using an MCF cut-off value of >68 mm with a sensitivity and specificity of 94%. FIBTEM MCF, with a cut-off of >24 mm, achieved only a sensitivity and specificity of 77% and 88%, respectively. This suggests that an elevated fibrinogen level which compensates for a low platelet count seems not to increase the thrombotic risk. The EXTEM and INTEM thrombodynamic potential index (TPI = (100 × MCF/100−MCF)/CFT), with a cut-off value of >3.5, provided even sensitivity and specificity of 100% and 92%, respectively. In conclusion, ROTEM® analysis was definitively superior to routine coagulation tests in identifying patients with thrombotic complications [107].

These results could be confirmed by another recently published prospective observational study in 318 noncardiac surgery patients. Here, Hincker et al. evaluated preoperative routine coagulation tests (aPTT, INR, and platelet count) and ROTEM® tests to identify patients at increased risk for postoperative thromboembolic complications; 29% of the included patient population has been recruited from the orthopedic and spine department. Again, none of the routine coagulation tests has been useful in predicting thrombo-

embolic events, but preoperative EXTEM and INTEM CFT, alpha angle, A10, and MCF were predictive for thromboembolic complications. INTEM and EXTEM A10 were the best predictors with a cut-off value of 61.5 mm and a ROC area under the curve (AUC) of 0.75 and 0.72, respectively. None of the FIBTEM parameters predicted thromboembolic complications, confirming that elevated fibrinogen levels alone seem not to be an independent risk factor for thrombosis [108].

Tissue Factor Expression on Monocytes

Stimulation with bacterial toxins, activation of purinergic (ADP) receptors ($P2X_7$), activated platelets, contact with surfaces of extracorporeal assist devices (e.g., cardiopulmonary bypass, ECMO, VADs, dialysis), as well as ischemia/reperfusion leads to TF-expression on circulating monocytes [28, 29, 109–115]. This TF-expression in the intravascular space results in delocalization/dissemination of coagulation and is an early and important pathomechanism of DIC and thrombosis. TF-expression on circulating monocytes can be detected very sensitively (in picomolar concentrations) but not specifically by a reduction in CT in NATEM [27, 29, 110, 115, 116]. Since heparinoids (e.g., by glycocalyx degradation or therapeutic administration) can mask this effect, heparinase should be added to the blood sample or test system (NA-HEPTEM) to eliminate a potential heparin effect [27].

Notably, TF-expressing monocytes inhibit fibrinolysis through a thrombin-activatable fibrinolytic inhibitor (TAFI)-mediated mechanism, which is the next step to microthrombosis and multiple organ failure [117].

Hypofibrinolysis/Fibrinolysis Shutdown

Physiologic fibrinolysis is shut down in the early phase of infection, sepsis, and thrombosis due to an upregulation of plasmin activator inhibitor type-1 (PAI-1) and activation of TAFI [30, 116–119]. Notably, whether the thrombin–thrombomodulin complex results in activation of protein C—with subsequent downregulation of PAI-1 and activation of fibrinolysis—or activation of TAFI—with subsequent shutdown of fibrinolysis—is regulated by platelet factor 4 (PF4) and dependent on the consumption of protein C as well as genetic polymorphisms [120–122]. Adamzik et al. showed that the ROTEM® LI60 in NA-HEPTEM can discriminate between intensive care patients suffering from severe sepsis (NA-HEPTEM LI60 $\geq$ 96.5% corresponding to an ML $\leq$ 3.5% within 1 h) and postoperative patients with systemic inflammatory response syndrome (SIRS) or healthy volunteers [27]. Furthermore, the LI60 (ROC AUC 0.901; $P < 0.001$) proved to be more accurate in the detection of sepsis than classical laboratory parameters such as procalcitonin (ROC AUC 0.75; $P < 0.001$). Interleukin-6 and C-reactive protein were not able to differentiate between septic and postoperative patients. The same research group also found that ROTEM findings were a better predictor of 30-day survival in sepsis patients than established risk scores (SAPS II, SOFA) [123]. Furthermore, Schmitt et al. showed that fibrinolysis shutdown occurs early in septic shock and is associated with increased morbidity and mortality in sepsis [124].

Also in TIC, Moore et al. could demonstrate that there are three different fibrinolytic phenotypes in trauma ad hospital admission [100, 125, 126]. Hypofibrinolysis or fibrinolysis shutdown (EXTEM LI60 > 98%) occurs in about 20% of patients in the early phase of severe trauma and is associated with increased mortality due to organ failure [127–129]. Physiologic fibrinolysis (EXTEM LI60, 82–97.9%) is with 70% the most common phenotype in the early phase of severe trauma and has the lowest mortality. Here, it is still under debate whether these patients should be treated with tranexamic acid (TXA) or not [130, 131]. Hyperfibrinolysis (EXTEM LI60 < 82%) occurs in about 10% of patients in the early phase of severe trauma and is associated with a very high mortality due to exsanguination—even if treated with TXA or not. Therefore, the corresponding ROTEM®/TEG® trace of fulminat fibrinolysis is also called the "death diamond" in trauma [132].

In conclusion, both hyperfibrinolysis and hypofibrinolysis/fibrinolysis shutdown seem to play an important role in the pathophysiology of TIC and DIC, and viscoelastic testing may be helpful in differentiating between both pathophysiologic entities and right decision-making regarding the appropriate use and timing of antifibrinolytic drugs. Notably, FIBTEM is the most sensitive and specific viscoelastic assay to detect fibrinolysis [57, 58]. Since the incorporation of alpha 2-antiplasmin into the clot needs activatable platelets and factor XIII, the FIBTEM clot is more sensitive to a plasmin attack [133]. Furthermore, platelet-mediated clot retraction—the differential diagnosis of low-grade fibrinolysis—does not occur in FIBTEM since the platelets are blocked in this assay [134].

Prediction of Mortality

Viscoelastic testing has been shown to be a good predictor of mortality in trauma [135]. Levrat et al. included 87 trauma patients in a prospective observational trial. Here, patients with hyperfibrinolysis who were more severely injured had greater coagulation abnormalities and a higher mortality rate (100% vs. 11%) [136]. Schöchl et al. identified in his database 33 patients with hyperfibrinolysis at hospital admission, retrospectively. They found hyperfibrinolysis to be a strong predictor for mortality (88%). Furthermore, it appeared that the earlier fibrinolysis could be detected by ROTEM®, the earlier the patient died, irrespective of appropriate treatment [137]. Theusinger et al. showed that in their patient population, mortality in the trauma hyperfibrinolysis group (77%), as diagnosed by ROTEM®, was significantly higher than in the non-trauma hyperfibrinolysis group (41%) and the matched trauma non-hyperfibrinolytic group (33%). Accordingly, hyperfibrinolysis was significantly ($P = 0.017$) associated with increased mortality in trauma [138].

In a prospective cohort study including 517 trauma patients, Rourke et al. found admission fibrinogen level to be an independent predictor of mortality at 24 h and 28 days. Hypofibrinogenemia could be detected early by FIBTEM A5 (A10), and administration of cryoprecipitate or fibrinogen concentrate could correct coagulopathy and improved survival [52]. Similar results were shown in a prospective cohort study in 334 blunt trauma patients performed by Tauber et al. They identified cut-off values of FIBTEM MCF < 7 mm, and EXTEM MCF < 45 mm as predictors for increased mortality. EXTEM MCF was independently associated with early mortality and hyperfibrinolysis increased fatality rates too [97].

Prolonged EXTEM CT and decreased EXTEM A10 (MCF) were associated with blood product transfusion, mortality, and disability at discharge in pediatric trauma patients too [139].

Thromboelastometry-Guided Hemostatic Therapy

Development of Thromboelastometry-Guided Algorithms

Pathophysiology of trauma-induced coagulopathy is complex and cannot always be addressed adequately by hemostatic resuscitation (1:1:1 concept) only (Fig. 18.6) [140–143]. In order to guide hemostatic therapy in bleeding patients, algorithms have been developed as a link between ROTEM® diagnostics and hemostatic therapy ("theranostic approach") [48, 142, 144–150]. These algorithms are based on the highest evidence actually available. The concept of a ROTEM®-guided bleeding management is to administer the right hemostatic drug/intervention, in the right dose, at the right time and the right sequence ("Precision Medicine"). Here, the high negative predictive value of ROTEM® is used to avoid any inappropriate blood transfusion or hemostatic intervention ("not-to-do" or "rule-out" algorithms) [48, 99]. Implementation of such algorithms has been shown to reduce transfusion requirements, complication rates, as well as morbidity and mortality in particular in cardiovascular surgery [151–156]. Several cohort studies reported similar results in trauma [52, 103, 135, 157–165], but RCTs are sparse in this field. However, Gonzalez et al. showed in a pragmatic RCT comparing viscoelastic to conventional

coagulation assays that utilization of a goal-directed, TEG-guided algorithm improved 28-day survival (19.6% versus 36.4%; $P = 0.049$) and decreased transfusion requirements for plasma and platelets [166]. Furthermore, Innerhofer et al. demonstrated in another RCT in severe trauma (RETIC trial) that ROTEM-guided coagulation factor concentrate (CFC) administration is much more effective to treat TIC compared to the transfusion of huge amounts of fresh frozen plasma (two times 15 mL per kg bodyweight) [167]. This study was terminated early for futility and safety reasons because of the high proportion of patients in the FFP group who required rescue therapy compared with those in the CFC group (52% in the FFP group vs 4% in the CFC group; odds ratio [OR] 25.34 [95% CI 5.47–240.03]; $P < 0.0001$) and increased need for massive transfusion (30% in the FFP group vs 12% in the CFC group; OR 3.04 [0.95–10.87]; $P = 0.042$) in the FFP group. Another safety aspect was the decreased incidence of multiple organ failure (50% versus 66%; $P = 0.15$) and venous thrombosis (8% versus 18%; $P = 0.22$) in the CFC group compared to the FFP group. Accordingly, Grottke and Rossaint mentioned in their comment that both, point-of-care coagulation monitoring and coagulation factor concentrates, might be essential for optimal treatment of TIC [168]. Results from further RCTs in severe trauma (STAT trial and iTACTIC trial) can be expected soon [169, 170].

A ROTEM®-guided A5 and A10 algorithms for bleeding management in trauma are presented in Fig. 18.7a, b. Since the ROTEM® parameter

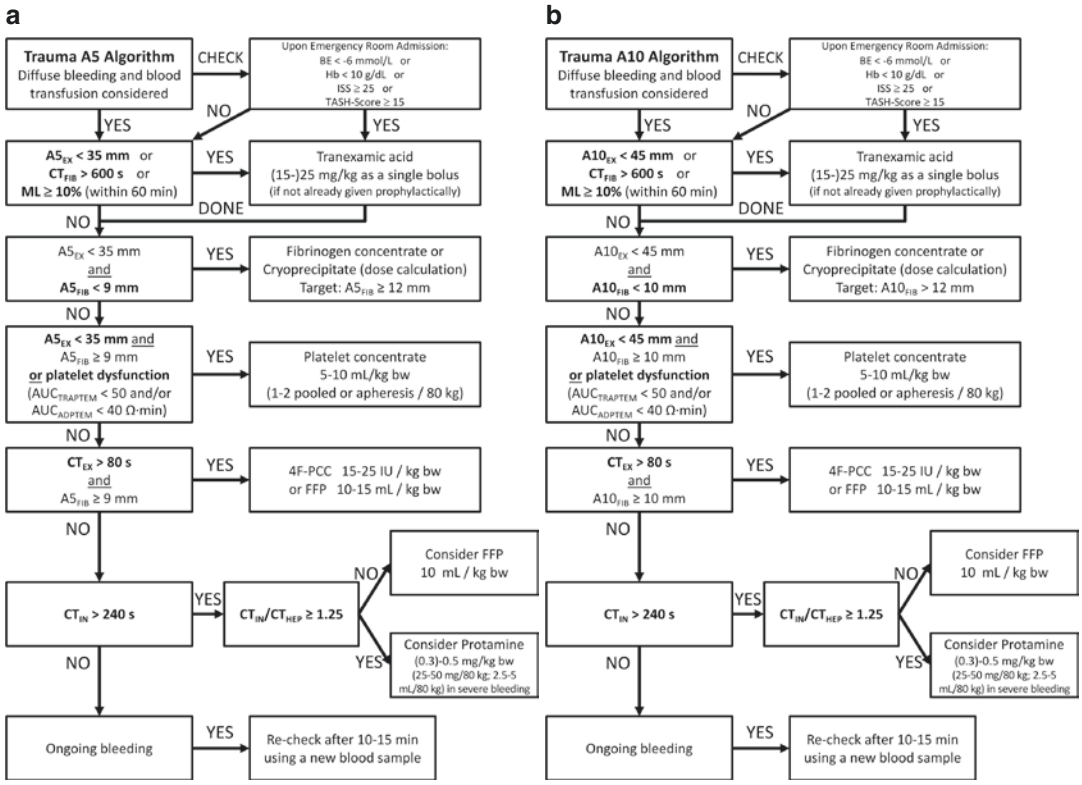


Fig. 18.7 A. Evidence-based ROTEM® A5 and B. A10 trauma algorithm. $A5_{EX}$ amplitude 5 min after CT in EXTEM, $A10_{EX}$ amplitude 10 min after CT in EXTEM, $A5_{FIB}$ amplitude 10 min after CT in FIBTEM, $A10_{FIB}$ amplitude 10 min after CT in FIBTEM, BE base excess, CT_{EX} coagulation time in EXTEM, CT_{FIB} coagulation time in FIBTEM (CT_{FIB} > 600 s reflects a flat-line in FIBTEM), CT_{HEP} coagulation time in HEPTEM, CT_{IN} coagulation time in INTEM, FFP fresh frozen plasma, Hb hemoglobin concentration, ISS injury severity score, ML maximum lysis (within 1 h run time), PCC prothrombin complex concentrate, TASH score trauma-associated severe hemorrhage score. (Courtesy of Klaus Görlinger, Essen, Germany)

A L G r a w a n y

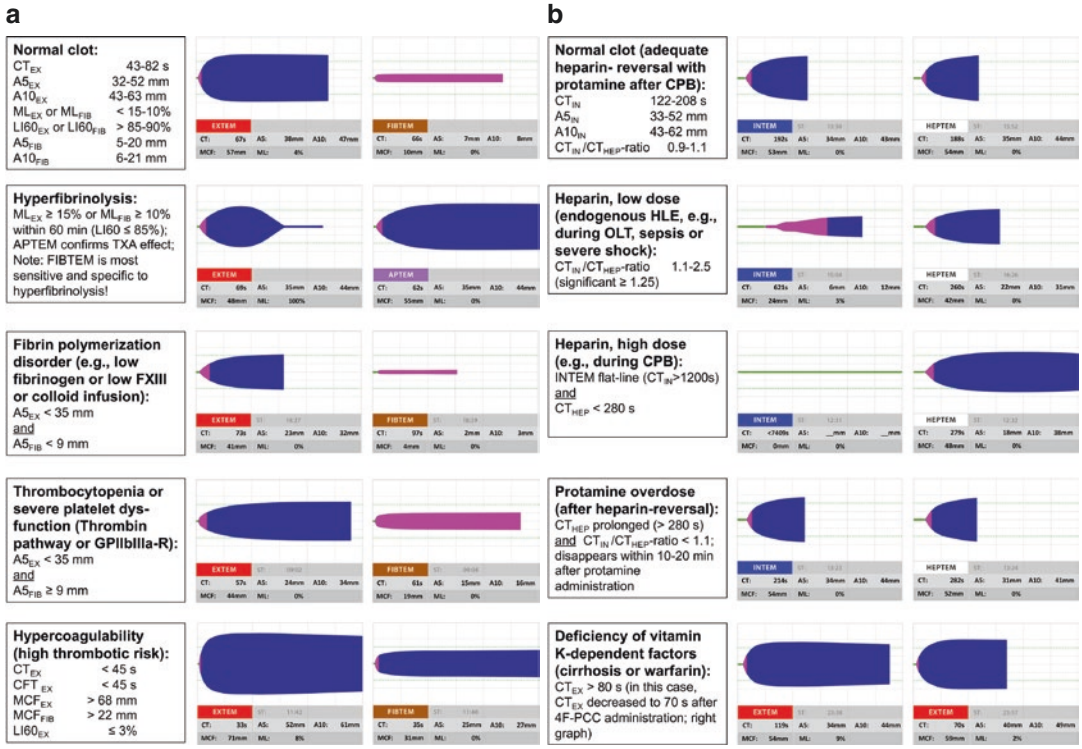


Fig. 18.8 Characteristic thromboelastometry (ROTEM® *delta/sigma*) traces. The diagnostic performance is increased by test combinations, e.g., EXTEM and FIBTEM, EXTEM and APTEM, or INTEM and HEPTEM. 4F-PCC four-factor prothrombin complex concentrate, CPB cardiopulmonary bypass, OLT orthotopic liver transplantation, TXA tranexamic acid (or other antifibrinolytic drug). (Courtesy of Klaus Görlinger, Essen, Germany)

A5 is not yet FDA-approved, ROTEM®-algorithms for the USA have to use A10, whereas A5 is used as a clot firmness parameter in the rest of the world in order to speed up decision-making. The difference between A10 and A5 for FIBTEM is 1 mm and for EXTEM, APTEM, INTEM, and HEPTEM 9–11 mm [49, 50]. Therefore, ROTEM® A5 and A10 algorithms can be transformed easily to each other. The characteristic thromboelastometric traces are presented in Fig. 18.8.

Clinical Assessment

Hemostatic interventions should be performed only in patients with diffuse bleeding and if blood transfusion is considered. The severity of trauma (ISS ≥ 25), clinical bleeding scores (e.g., TASH score ≥ 15), hypothermia (core temperature < 35 °C), and results of blood gas analysis (e.g., pH < 7.2, BE <−6 mmol/L, Hb < 10 g/dL, Ca^{i++} < 1 mmol/L) should be considered, too, and may be associated with an increased risk of hyperfibrinolysis and hypofibrinogenemia [93, 171–173]. However, decision-making for hemostatic interventions should not be based on ROTEM® results, solely, in the absence of clinically relevant bleeding ("Don't treat numbers!") [48].

Management of Fibrinolysis

Hyperfibrinolysis (ML > 18% within 60 min corresponding to a LI60 < 82%) is associated with increased mortality (53% vs. 4%) due to bleeding in severe trauma compared to physiologic fibrinolysis (ML, 2–18% within 60 min corresponding to a LI60, 82–98%) [100, 126]. However, hypofibrinolysis/fibrinolytic shutdown (ML < 2% within

60 min corresponding to a LI60 > 98%) is also associated with increased mortality (26% vs. 4%) due to organ failure in severe trauma [100, 126, 174]. This is in line with the data published by Adamzik et al. and Schmitt et al. that fibrinolysis ≤3.5% 1 h after CT (LI60 ≥ 96.5%) at ICU admission is associated with increased mortality in sepsis [27, 123, 124]. Therefore, exogenous inhibition of the fibrinolysis system in severely injured patients requires careful selection, as it may have an adverse effect on survival, in particular if tranexamic acid is given later than 3 h after injury [104–106, 126, 130, 174–181]. In order to enable quick decision-making, early thromboelastometric variables of clot firmness in EXTEM (A5 and A10) can be used to identify patients at risk for fibrinolysis. Here, an EXTEM A5 threshold of <35 mm (EXTEM A10 < 45 mm) detects more than 90% of patients with hyperfibrinolysis [54]. Colloid infusion (HES > gelatin) results in reduced resistance of polymerized fibrin to plasmin degradation [182]. In contrast, high factor XIII levels attenuate tissue plasminogen activator-induced hyperfibrinolysis in human whole blood [133]. Furthermore, a flat-line in FIBTEM characterized by a FIBTEM CT > 600 s seems to be associated with hyperfibrinolysis too.

Management of Clot Firmness

Trauma-induced coagulopathy is functionally characterized by a reduced clot firmness in EXTEM with an A5 < 35 mm or an A10 < 45 mm, respectively, and predicts the need for massive transfusion [55, 98, 99, 150]. Here, reduced clot firmness can be based on hypofibrinogenemia, fibrin polymerization disorders (e.g., due to colloids), thrombocytopenia, and severe thrombocytopathy (reduced platelet aggregation due to activation of platelets' thrombin receptors).

FIBTEM A5 (A10) can be used for rapid and correct discrimination between hypofibrinogenemia and thrombocytopenia [25, 51–53, 96]. Here, a FIBTEM A5 < 9 mm or an A10 < 10 mm is associated with an increased risk of massive transfusion and can be used as a trigger value for fibrinogen substitution [96, 98, 150]. The optimum target value seems to be ≥12 mm. However, some patients may even need a higher targeted value of 16 mm—in particular in patients with severe bleeding due to unstable pelvic fractures, liver injury, and traumatic brain injury [183, 184].

The fibrinogen dose can be calculated based on the targeted increase in FIBTEM A5 (A10):

$$\text{Fibrinogen dose}\,(\text{g}) = \text{targeted increase in FIBTEM A5}\,(\text{mm}) \times \text{body weight}\,(\text{kg})\,/\,160$$

Here, the correction factor (140–160 mm kg g^{-1}) depends on the actual plasma volume [52, 159, 171, 185–191]. Notably, the achieved increase may be lower than the calculated increase in severe bleeding due to ongoing fibrinogen consumption. Fibrinogen substitution can be done by fibrinogen concentrate administration or cryoprecipitate transfusion, dependent on the local approval and availability. Table 18.5 provides a quick overview of the fibrinogen dose needed to achieve the targeted increase in FIBTEM A5 (A10).

If clot firmness in EXTEM is reduced (A5 < 35 mm or A10 < 45 mm) but FIBTEM clot firmness is above the trigger value (A5 ≥ 9 mm or A10 ≥ 10 mm), platelet transfusion has to be considered in bleeding trauma patients. Notably, ROTEM® analysis has been shown to be superior to platelet count in the prediction of bleeding in patients with severe thrombocytopenia [192]. The expected increase in EXTEM A5 (A10) per transfused pooled or apheresis platelet concentrate is 5–10 mm in adult patients [193, 194]. Therefore, the number of transfused platelet concentrates can be calculated based on the targeted increase in EXTEM A5 (A10).

Notably, standard viscoelastic assays are not sensitive to the effects of antiplatelet drugs such as COX-inhibitors (e.g., aspirin) and ADP-receptor antagonists (e.g., clopidogrel, prasug-

Table 18.5 FIBTEM-guided fibrinogen substitution

Targeted increase in FIBTEM A5 (A10) (mm)	Fibrinogen dose (mg/kg bw)	fibrinogen concentrate (mL/kg bw)	Cryoprecipitate (mL/kg bw)
2	12.5	0.6 [1 g per 80 kg]	1 [5 U per 80 kg]
4	25	1.2 [2 g per 80 kg]	2 [10 U per 80 kg]
6	37.5	1.9 [3 g per 80 kg]	3 [15 U per 80 kg]
8	50	2.5 [4 g per 80 kg]	4 [20 U per 80 kg]
10	62.5	3.1 [5 g per 80 kg]	5 [25 U per 80 kg]
12	75	3.8 [6 g per 80 kg]	6 [30 U per 80 kg]

Here, fibrinogen dose calculation is based on the targeted increase in FIBTEM A5 (A10) in mm. In case of severe bleeding, the achieved increase in FIBTEM A10 (A5) may be lower than the calculated increase. Courtesy of Klaus Görlinger, Essen, Germany

rel, and ticagrelor) since high amounts of thrombin are generated in the test system which overcomes the effects of antiplatelet drugs. Therefore, platelet function analysis should be performed in patients with suspected platelet dysfunction. In the ROTEM system, this is realized by the ROTEM® *platelet* module (CE-marked in Europe since November 2013), which provides two channels of whole blood impedance aggregometry in addition to the four viscoelastic channels of the ROTEM® *delta* device (Fig. 18.1). Whole blood impedance aggregometry provides rapid and reliable data about platelet function within 10 min (Fig. 18.9). Besides the detection of the effects of antiplatelet drugs and other drugs with antiplatelet effects (e.g., analgetics, antidepressants, antibiotics, cardiovascular drugs), whole blood impedance aggregometry has been shown to detect early direct effects of trauma and sepsis

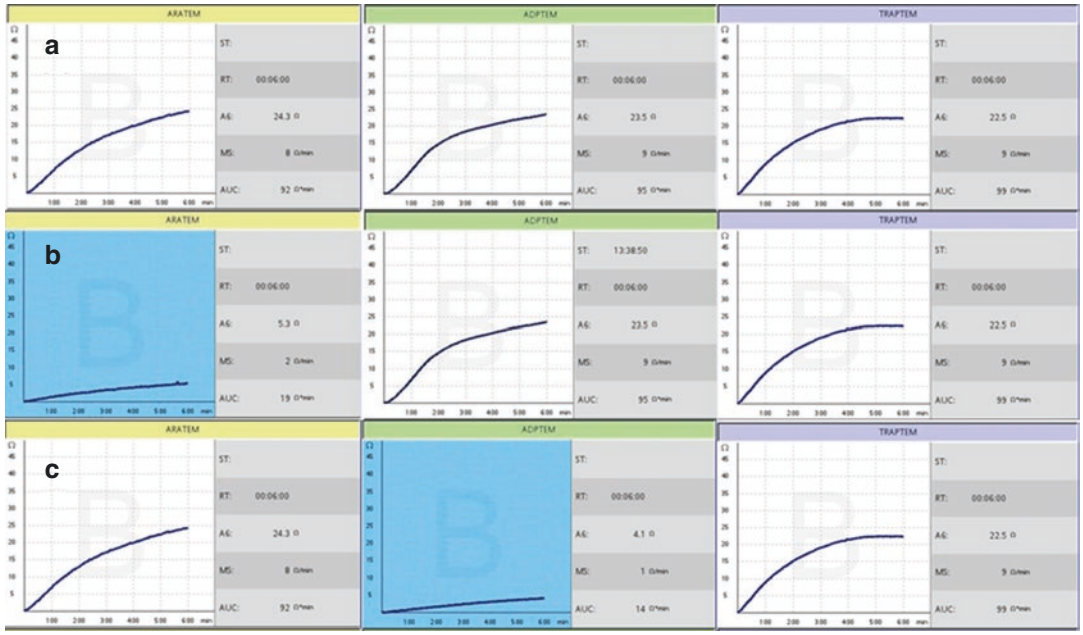


Fig. 18.9 Characteristic whole blood impedance aggregometry traces (ROTEM® *platelet*) achieved by activation with arachidonic acid (ARATEM; *left column*), ADP (ADPTEM; *middle column*), and TRAP-6 (TRAPTEM; *right column*). *Line A*: normal platelet function; *Line B*: selective inhibition of the arachidonic acid pathway (e.g., by aspirin); *Line C*: selective inhibition of the ADP-receptor pathway (e.g., by clopidogrel or prasugrel); *Line D*: inhibition of the arachidonic acid and ADP-receptor pathway (e.g., dual antiplatelet therapy with aspirin and clopidogrel); *Line E*: selective inhibition of the thrombin-receptor pathway (e.g., by vorapaxar); *Line F*: general platelet dysfunction due to triple antiplatelet therapy, GPIIbIIIa-receptor antagonists (e.g., abciximab, eptifibatide, or tirofiban), platelet receptor destruction (e.g., due to cardiopulmonary bypass, severe trauma, or sepsis), or severe thrombocytopenia. (Courtesy of Klaus Görlinger, Essen, Germany)

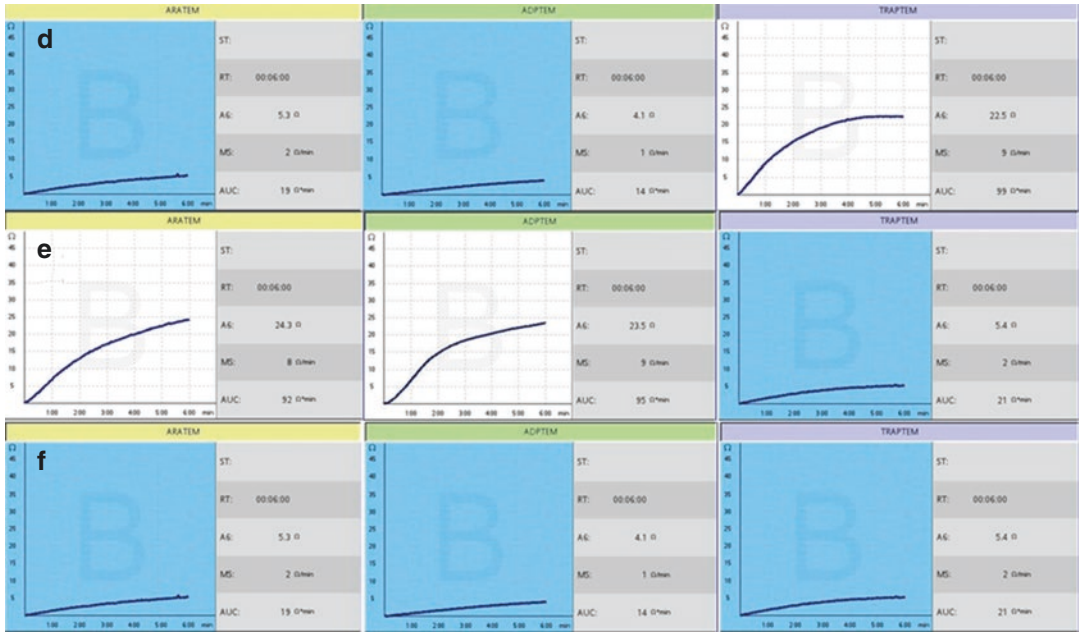


Fig. 18.9 (continued)

on platelet function which is associated with increased mortality [38, 68, 71–74, 76, 85, 195–197]. However, actually, it is not yet clear whether early trauma-induced platelet dysfunction should be treated with platelet transfusion or not [82, 198].

Management of Coagulation Time (Thrombin Generation)

Coagulation times (CT) can be prolonged due to a deficiency of enzymatic coagulation factors or the presence of an anticoagulant, for example, warfarin, heparin, or direct oral anticoagulants (DOACs) [199, 200]. A CT prolongation in EXTEM solely indicates a deficiency of vitamin K-dependent coagulation factors (factor VII, X, II; extrinsic pathway) due to a therapy with vitamin K-antagonists (warfarin) or hemodilution/consumption. Usually, the activity of the vitamin K-dependent coagulation factors is decreased below 30% of their normal activity if CT in EXTEM exceeds 80s [159, 201, 202]. However, a severe fibrinogen deficiency can prolong CT in EXTEM too. Therefore, EXTEM CT can be used for guiding therapy with prothrombin complex concentrate (PCC) or fresh frozen plasma (FFP) only in case of a normal A5 (A10) in FIBTEM. Accordingly, management of clot firmness precedes the management of coagulation time in the ROTEM algorithm. Here, the use of three- or four-factor PCCs or FFP is dependent on the local approval and availability in the respective countries. Notably, four-factor PCCs (Beriplex® and Octaplex®) are approved in Europe for prophylaxis and therapy of bleeding in patients with hereditary and acquired deficiencies of vitamin K-dependent factors, whereas four-factor PCC (Kcentra®) in the USA is FDA-approved for urgent reversal of vitamin-K antagonists only. Four-factor PCCs have the advantage of being a standardized product enabling a rapid and calculated increase in coagulation factor activity and avoiding transfusion-associated circulatory overload (TACO), transfusion-related lung injury (TRALI), and transfusion-related immunomodulation (TRIM) which are typical and serious complications of FFP transfusion [86, 157–159, 203–210]. Notably, direct thrombin inhibitors such as dabigatran can result in a marked increase in EXTEM and INTEM CT as well as in ECATEM CT [33, 34, 199, 200, 210–212]. Here, the ecarin-based ROTEM assay

ECATEM is specific for direct thrombin inhibitors such as hirudin, argatroban, bivalirudin, and dabigatran [31–33, 213].

Due to the high risk of arterial thromboembolic events, the off-label administration of rFVIIa should be restricted to bleeding not responding to comprehensive coagulation therapy [214, 215]. Activated PCCs (Factor Eight Inhibitor Bypassing Agent = FEIBA) are not indicated in trauma-induced coagulopathy too. The implementation of thromboelastometry-guided bleeding management algorithms usually eliminates the need for rFVIIa administration as a rescue therapy [151, 152, 216, 217].

A prolongation of INTEM CT can be based on a heparin effect or a deficiency of coagulation factors of the intrinsic pathway. A clinically relevant heparin effect, for example, due to endothelial glycocalyx degradation or re-transfusion of heparin by using a cell-saver in the emergency modus, can be confirmed by an INTEM/HEPTEM CT-ratio ≥ 1.25 [11, 22, 218]. Here, protamine administration can be considered. In case of prolongation of CT in INTEM and HEPTEM, FFP transfusion can be considered in bleeding trauma patients.

Clinical and ROTEM® Reassessment

Finally, clinical bleeding has to be reassessed after running through the algorithm. In case of ongoing bleeding, ROTEM® has to be reassessed with a new blood sample and the algorithm has to be started again. In the RETIC trial, hemostatic could be achieved after two rounds of the ROTEM-guided algorithm in 96% of the trauma patients [167].

Thromboelastometry-Guided Bleeding Management Algorithms—Impact on Outcome

Implementation of ROTEM®-guided bleeding management algorithms reduced bleeding and transfusion requirements in several clinical settings including severe trauma [135, 153, 155, 162].

Görlinger, Fries, and Schöchl reported in their retrospective analysis that implementation of a ROTEM®-guided therapy with coagulation factor concentrates reduced transfusion requirements for FFP by 88–94% in their institutions in different perioperative settings (trauma, visceral, and transplant surgery and cardiovascular surgery). At the same time, the incidence of intraoperative massive transfusion (≥10 units of RBCs) could more than halved (1% vs. 2.5%; $P < 0.001$), and RBC and platelet transfusion requirements could be reduced significantly by 8–62% and 21–72%, respectively [159].

Schöchl et al. analyzed transfusion incidence for RBCs and platelets in the emergency room and during primary surgery in a retrospective study including 80 trauma patients treated at the trauma hospital AUVA in Salzburg, Austria, with a thromboelastometry-guided coagulation factor concentrate group versus 601 patients from the Trauma Register DGU, Germany, with standard FFP-based therapy (≥2 units FFP; median 6 units FFP; range 2–51). Here, the transfusion incidence for RBCs (71% vs. 97%; $P < 0.001$) and platelets (9% vs. 44%; $P < 0.001$) was significantly lower in the thromboelastometry/factor concentrate group compared to the standard FFP group [158].

Nienaber et al. analyzed the incidence of multiple organ failure (MOF) in a matched-pair analysis including 18 severely injured and bleeding trauma patients from the Innsbruck Trauma Database (ITB), Austria, treated according to a ROTEM®-guided coagulation factor concentrate protocol without FFP and 18 patients from the Trauma Register DGU, Germany, treated with an FFP protocol without coagulation factor concentrates. Here, the incidence of MOF was significantly lower in the ROTEM®/factor concentrate group compared to the FFP group (16.7% vs. 61.1%; $P = 0.015$) [220].

In a prospective cohort study including 517 trauma patients, Rourke et al. found that hypofibrinogenemia could be detected early by FIBTEM A5 (A10), and administration of cryoprecipitate or fibrinogen concentrate could correct coagulopathy and improved survival [52].

Schöchl et al. performed a retrospective analysis including 131 trauma patients who received

≥5 units of RBC concentrates within 24 h, and TIC was managed by ROTEM®-guided treatment with coagulation factor concentrates. After excluding 17 patients with traumatic brain injury, the mortality observed in this study was significantly lower (14%) than predicted by the trauma injury severity score (TRISS; 27.8%; $P = 0.002$) and the revised injury severity classification score (RISC ; 24.3; $P = 0.014$) [157].

Lendemans et al. reported in a retrospective study before and after implementation of a ROTEM-guided algorithm in 172 trauma patients with an ISS > 16 a significant reduction in multiple organ failure (36.3% vs. 66.7%; $P = 0.0001$) and in-hospital mortality (20.9% vs. 38.3%; $P = 0.012$). In this study, performed in cooperation with the Trauma Register of the German Trauma Society (DGU) and supported by the German Interdisciplinary Association of Critical Care and Emergency Medicine (DIVI), 24 h mortality was reduced by 50% (9.9% vs. 19.8%), too, but did not reach statistical significance [161].

Stein et al. demonstrated that the implementation of a ROTEM®-guided coagulation management in 731 major trauma patients was associated with a reduced incidence in massive transfusion (4% vs. 12%; $P < 0.001$), FFP transfusion in the emergency department (5% vs. 31%; $P < 0.001$), and overall mortality (22% vs. 33%; ($P < 0.001$). In bleeding trauma patients, it is essential that the initial ROTEM® analysis is performed in the emergency department as soon as the patient arrives [10, 163, 219].

Besides reduction in transfusion requirements, ROTEM®-guided bleeding management in particular in cardiovascular surgery has been shown to reduce complication rates as well as morbidity and mortality in cohort studies and RCTs [151–153, 155, 159]. As shown above (see also section "Development of Thromboelastometry-Guided Algorithms"), several cohort studies and one RCT (RETIC trial) reported similar results in trauma, but data from RCTs are still sparse in trauma [52, 97, 135, 157–163, 167, 220]. However, results from the STAT trial and iTACTIC trial can be expected soon [169, 170].

Therapeutic Window Concept

The algorithms presented here are based on the "therapeutic window concept." This concept has been developed for guiding antiplatelet therapy in patients undergoing percutaneous coronary interventions (PCIs) in order to minimize the risk of ischemia (stent thrombosis) and bleeding [221, 222]. Accordingly, bleeding management algorithms guided by thromboelastometry and whole blood impedance aggregometry are designed to minimize the risk of both bleeding and thrombosis by an individualized therapy. Here, the right therapeutic intervention, in the right dose and the right sequence, is defining the framework of the therapeutic window, for example:

- EXTEM A5 (A10): 35–45 mm (45–55 mm)
- FIBTEM A5 (A10): 9–15 mm (10–16 mm)
- EXTEM CT: 60–80 s
- ADPTEM AUC: 40–120 Ω·min
- TRAPTEM AUC: 50–150 Ω· min

Using this concept in cardiovascular surgery, it was possible to reduce both transfusion requirements and thrombotic/thromboembolic complications significantly [151, 152, 154, 223].

Accordingly, ROTEM®-guided coagulation factor concentrate (CFC) administration in the RETIC RCT resulted, on the one hand, in a significant decrease in the needed for massive transfusion (12% in the CFC group vs. 30% in the FFP group; OR 3.04 [0.95–10.87] $P = 0.042$) and, on the other hand, in a decrease in the incidence of venous thrombosis (8% versus 18%; $P = 0.22$) [167].

Guidelines, Health Technology Assessments, Knowledge Translation, and Implementation

Based on the actually available evidence, the implementation of ROTEM®-guided algorithms is highly recommended (Grade 1B) by the updated guidelines for the management of severe perioperative bleeding from the European Society of

Anesthesiology (ESA) and the updated European guideline for the management of bleeding and coagulopathy following major trauma [224, 225]. In particular, therapy with coagulation factor concentrates, such as fibrinogen concentrate and prothrombin complex concentrate (PCC), should be guided by thromboelastometry (Grade 1C). Furthermore, it is stated that the implementation of transfusion and coagulation management algorithms (based on ROTEM®/TEG®) can reduce transfusion-associated costs in trauma, cardiac surgery and liver transplantation (Grade B), and that targeted therapy with fibrinogen and/or PCC guided by ROTEM®/TEG® is not associated with an increased incidence of thromboembolic events (Grade C) [224].

The cost-effectiveness of ROTEM®-guided bleeding management has also been proven by several health technology assessments and pharmaco-economic analyses [226–233]. However, guidelines and heath technology assessments can only change practice and improve patients' outcomes in combination with knowledge translation and implementation [230–233]. Therefore, the 'STOP Bleeding Campaign' was set up in 2013 [234].

Dedication This chapter is dedicated to my wife Dr. Anke Görlinger, who passed away much too early on December 27, 2019. Without her support, this work could not have been done.

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19 Thromboelastography (TEG® 5000 and TEG® 6s Hemostasis Analyzers with TEG Manager® Software)

Jan Hartmann and Robert A. Sikorski

Abbreviations

AA	Arachidonic acid
ACT	Activated clotting time
ADP	Adenosine diphosphate
aPTT	Activated partial thromboplastin time
CABG	Coronary artery bypass grafting
CCT	Conventional coagulation test
CFF	Citrated functional fibrinogen
CK	Citrated kaolin
CRT	Citrated RapidTEG™
DOACs	Direct-acting oral anticoagulants
FDP	Fibrin degradation product
FF	Functional fibrinogen
FLEV	Level of fibrinogen in the plasma
INR	International normalized ratio
K-time	Coagulation time
LY30	Percent lysis 30 minutes after maximum amplitude
MA	Maximum amplitude
PCI	Percutaneous coronary intervention
POCT	Point-of-care test
PPH	Postpartum hemorrhage
PT	Prothrombin time
R-time	Reaction time
TAVR	Transcatheter aortic valve repair/replacement
TT	Thrombin time
TXA	Tranexamic acid
VHA	Viscoelastic hemostatic assays

Introduction

Analyzer Methodology

Many technicians and physicians are familiar with using the TEG® 5000 system for performing thromboelastography. Whole blood (sample volume ~ 360 μL; temperature 37 °C) is transferred to a cylindrical cup inserted into the device along with reagents for the assay to be performed. A stationary pin is immersed into the blood, and an oscillating rotational movement is introduced whereby the cup is rotated through an angle of 4° 45′ with a cycle duration of 10 seconds [1]. These conditions are designed to reflect venous blood flow.

At the outset, the blood is a liquid with low viscosity; there is no measurable rotational force on the pin and the read-out on the TEG® 5000 device is zero. As the process of coagulation begins, fibrin fibers begin to form within the blood; these adhere to the pin and the cup while increasing the viscosity of the blood. As a result,

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the rotation of the cup begins to exert a rotational force on the pin. The TEG® 5000 device registers values that represent the extent of the pin's rotational movement (i.e., the amplitude), which increases with the rotational force. The amplitude increases over time until a maximum is reached, and a value for maximum amplitude (MA) is obtained. Subsequently, the amplitude decreases gradually as the natural process of clot lysis takes place [1].

The results of a thromboelastography analysis are presented both as specific values (e.g., reaction time, MA [see below for details of the variables that are measured]) and as a graph of amplitude (in millimeters; y-axis) against time (in minutes; x-axis). A blood sample from a healthy individual will yield a graph (tracing) that has a profile similar to a cognac glass on its side, as coagulation proceeds more quickly than clot lysis.

The TEG® 6s system was designed to advance the ease and precision of thromboelastography, and is increasingly being used in place of the TEG® 5000. Both devices are shown in Fig. 19.1. The TEG® 6s device is used with disposable cartridges that contain all of the assay reagents, avoiding the need for reagents to be pipetted manually. A sample of citrated blood (~340 μL) is pipetted into the cartridge, triggering an automated process that mixes the assay reagents with the blood and transfers them to the test chamber within the device. While similar parameters are measured as with the TEG® 5000 device, a different method is used to assess coagulation: instead of rotating a cup and measuring the forces involved, the sample is exposed to vibrations of different frequencies to identify the resonant frequency. Viscoelastic properties (and therefore clot strength) affect the resonant frequency in a predictable manner, enabling the same parameters and tracing of the TEG® 5000 device to be reproduced. TEG® 6s analyzer results are therefore interpreted clinically in the same way as TEG® 5000 data. Importantly, the increased automation with TEG® 6s limits the potential for operator-dependent variability while minimizing the time taken for results to become available [1].

The software used with TEG® 6s Hemostasis Analyzer is called TEG Manager®, and is another area where advances have been made. The results of each TEG® 6s analysis are uploaded to a server-based Web portal. Physicians can review their current and historical data from anywhere within the hospital network at any time, and hospital administrators as well as laboratory managers and directors can monitor all the devices within their jurisdiction to facilitate managerial decisions. Password protection ensures data safety and that the software is fully compliant with the Health Insurance Portability and Accountability Act [1]. A separate interpretation guidance module allows for institutions to define and set their own cut-offs and algorithms, which can facilitate a more aligned approach across larger teams of providers (Fig. 19.2).



Fig. 19.1 The TEG® 5000 (left) and TEG® 6s (right) devices. TEG® Hemostasis Analyzer Operator tracings and images used by permission of Haemonetics Corporation

TEG® Tracing and Variables Measured

A number of different thromboelastography variables are used to assess the clotting process, all of which are based on changes in clot strength over time. Details of the variables most likely to be used in clinical practice are described below.

R-Time

The reaction time (R) is the time it takes for the amplitude of the clot tracing to reach 2 mm. It reflects the process of thrombin generation and

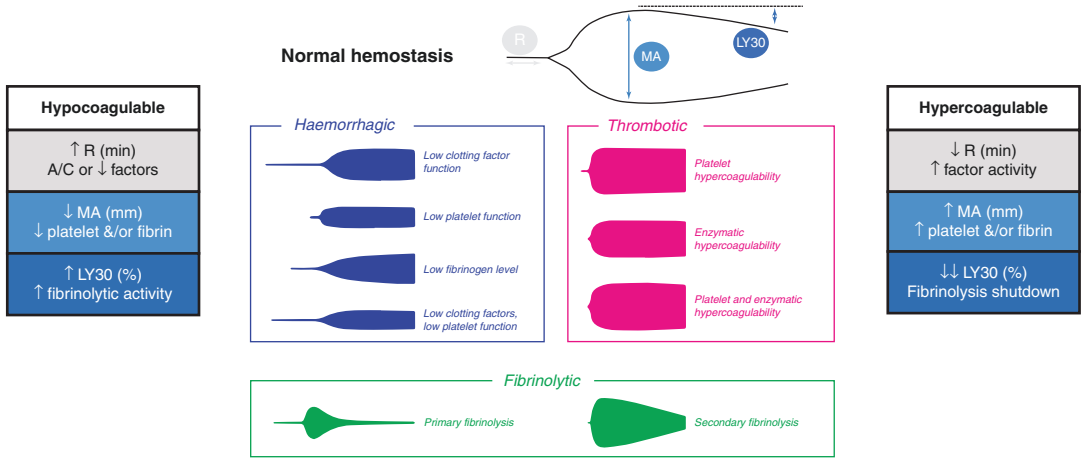


Fig. 19.2 Examples of TEG® traces for normal hemostasis, hemorrhagic, thrombotic, or fibrinolytic states. A/C anticoagulant, LY30 percent lysis 30 minutes after MA, MA maximum amplitude, R reaction time. TEG® Hemostasis Analyzer Operator tracings and images used by permission of Haemonetics Corporation

the initial formation of fibrin fibers. Conditions that can increase the value of R-time are those associated with hypocoagulability, such as clotting factor deficiencies or treatment with anticoagulants (e.g., heparin) [1, 2]. Conversely, hypercoagulability may cause a low value for R-time. Nielsen et al. [3] studied the effects of specific coagulation factor deficiencies (II, VII, X, XII) on thromboelastography results, and reported that R-time was the variable most affected by these deficiencies. Also, in patients with hemophilia, R-time has shown a strong correlation with factor VIII activity ($R = -0.81$, $p = 0.001$) [4].

K-Time

The coagulation time (K) is defined as the time interval between the tracing reaching amplitudes of 2 and 20 mm. This variable represents the process of clot strengthening, which is mainly dependent on the cleavage of fibrinogen and subsequent polymerization of fibrin. As with R-time, a prolonged value for K-time is often encountered under conditions of hypocoagulability, while hypercoagulability can shorten K-time [1]. Severe coagulopathy may prevent the tracing amplitude from reaching 20 mm, meaning that no value for K-time is produced. Consequently, K-time is used less in clinical practice than the other variables described here, particularly as the functional fibrinogen assay is included in the TEG® 6s device.

Angle

The angle (α) is the tangent line of the tracing that starts as the tracing diverges from the baseline. This variable reflects the speed of clotting and depends on coagulation factors including fibrinogen. An angle that is smaller than the reference range suggests that the patient is in a hypocoagulable state, while a larger angle is likely to be encountered in hypercoagulable patients. Angle is used in some clinical algorithms to determine a patient's need for fibrinogen supplementation, on the basis that it correlates well with functional fibrinogen concentrations ($R = 0.7$, $p < 0.0001$) [5]. However, amplitude in the Functional Fibrinogen TEG (TEG-FF) assay may be preferable for such assessment, as platelet aggregation is inhibited in the TEG-FF assay.

Maximum Amplitude (MA)

The maximum amplitude (MA) is the most divergent point of the tracing, representing the maximum strength of the clot. This depends largely on the concentrations and functionality of platelets and fibrin. Thus, MA has been shown to correlate with both platelet count ($r = 0.59$, $p < 0.001$) and fibrinogen level ($r = 0.64$, $p < 0.001$) [6]. The amplitude at 10 minutes has been suggested as a

parameter to provide an early indication of MA, but it is currently only available outside the United States [7].

LY30

The LY30 parameter describes the percentage reduction in the area under the TEG® tracing at 30 minutes after the MA was achieved, compared with the area under a hypothetical constant tracing from the time of MA. LY30 is an assessment of clot stability, and shows the speed and extent of fibrinolysis (high values are associated with hyperfibrinolysis). In a study of trauma patients, LY30 values ≥3% as opposed to <3% were associated with a significantly higher likelihood of needing massive transfusion (90.9% vs. 30.5%, $p = 0.0008$) and an increased risk of death due to bleeding (45.5% vs. 4.8%, $p = 0.0014$), particularly in patients with a short time to maximum amplitude (TMA) [8].

Reference Ranges

Reference ranges for TEG® measurements most likely to be used in clinical practice are shown in Table 19.1. The values shown are based on tests of citrated whole blood from healthy donors representative of normal population distributions of age, gender, and race. The donors had no known coagulopathies and were not taking any drugs that would potentially affect hemostasis. A recent method comparison study found a strong correlation between TEG® 5000 and TEG® 6s measurements, with very similar reference ranges [9].

Table 19.1 Reference ranges for TEG® 6s Hemostasis Analyzer parameters

Reagent and variable	Reference range: lower boundary	Reference range: upper boundary
CK R (minutes)	4.6	9.1
CK LY30 (%)	0.0	2.6
CRT MA (mm)	52	70
CFF MA (mm)	15	32

CFF citrated functional fibrinogen, *CK* citrated kaolin, *CRT* Citrated RapidTEG®, *MA* maximum amplitude, *R* reaction time

Clinical Interpretation of TEG® Results

The interpretation of TEG® results requires experience because of the number of variables involved (due to both user variability and sample variability), and the fact that most of the relevant variables can be affected by more than one aspect of coagulation. Table 19.2 shows TEG® results likely to be observed in the clinic, and how these results may be interpreted. The guidance shown is based on in vitro studies where specific adjustments could be made (e.g., inhibition of platelet function) and the effects on TEG® results observed.

Channels and Reagents

Thromboelastography can be performed using a number of assays with different reagents, enabling different aspects of coagulation to be assessed. The TEG® 5000 device provided two channels, allowing two assays to be performed simultaneously; with the TEG® 6s, the number of channels has been increased to four, so that four assays can be run simultaneously without the need for reagent preparation. Details of the principal TEG® assays are detailed below and summarized in Table 19.3.

Native

This assay is performed without any additional reagents and therefore characterizes the coagulation process by contact activation. This assay is more likely to be used for research purposes than in routine clinical practice, because, in the absence of a coagulation activator, the clotting process is relatively slow and results are not available quickly.

RapidTEG™

Two coagulation activators are used in this assay: kaolin and human recombinant tissue factor. These two reagents have complementary modes of action; kaolin stimulates coagulation via the intrinsic pathway while tissue factor activates the extrinsic coagulation cascade. For RapidTEG, activated clotting time (ACT) measures the speed of the early phase of coagulation and serves as a

Table 19.2 Interpretation guidance for TEG® 6s Hemostasis Analyzer results

Assay	Variable	Observed TEG result	Hemostatic significance	Interpretation
CK	R (min)	CK R > RR	Hypocoagulable	Low coagulation factor activity and/or presence of heparin
		CK R < RR	Hypercoagulable	
	LY30 (%)	CK LY30 > RR	Hypocoagulable	Hyperfibrinolysis
CRT	MA (mm)	CRT MA < RR	Hypocoagulable	Low fibrinogen level or low platelet contribution
		CRT MA > RR	Hypercoagulable	High platelet contribution
CFF	MA (mm)	CFF MA < RR	Hypocoagulable	Low fibrinogen level
		CFF MA > RR	Hypercoagulable	High fibrinogen level

CFF citrated functional fibrinogen, *CK* citrated kaolin, *CRT* Citrated RapidTEG, *MA* maximum amplitude, *R* reaction time

Table 19.3 Key assays and reagents for TEG® 5000 and TEG® 6s Hemostasis Analyzers

Assay	Reagents
Native	No additional reagents
RapidTEG	TF, kaolin and $CaCl_2$
Kaolin	Kaolin and $CaCl_2$
Heparinase	Kaolin, $CaCl_2$, and heparinase
FF	Abciximab, tissue factor, and $CaCl_2$
Platelet mapping	Four separate assays: Kaolin assay – as above ActivatorF assay – heparin, reptilase, and factor XIIIa AA plus ActivatorF assay – heparin, reptilase, factor XIIIa, and AA ADP plus ActivatorF assay – heparin, reptilase, factor XIIIa, and ADP

AA arachidonic acid, *ADP* adenosine diphosphate, *FF* functional fibrinogen, *TF* tissue factor

replacement for R-time. A value for ACT is produced as early as 3 minutes after beginning the assay, enabling key treatment decisions to be made early. MA and Angle are available within ~15 minutes.

Kaolin

The Kaolin assay is widely used in clinical settings where the speed offered by Rapid TEG™ is not required. Kaolin is the sole activator of coagulation in this assay. It is sometimes regarded as the 'standard' TEG® assay, serving as a baseline for comparison with assays with additional reagents. The Kaolin assay is usually performed using citrated whole blood, as this prevents the blood from clotting prematurely.

Heparinase

The Heparinase assay is performed to determine whether clinically relevant levels of heparin are present in the patient's blood. Most commonly, the results of a kaolin–heparinase assay are compared with those of the Kaolin assay. Typically, users compare R-times to determine if heparin is present, and if present, use the MA on the citrated kaolin with heparinase channel. In addition to the Kaolin assay, the effect of heparinase can be measured using the native, citrated functional fibrinogen (CFF) and Rapid TEG™ assays (i.e., these two assays can also be performed with versus without heparinase).

Functional Fibrinogen (FF)

The contribution of fibrinogen to the strength of the clot can be measured using the Functional Fibrinogen (FF) assay. The overall clot strength is derived from two sources: platelets and fibrin, the latter being derived from fibrinogen. The FF assay is performed with a platelet glycoprotein IIb/IIIa receptor antagonist, which prevents platelets from binding and contributing to the clot formation. Consequently, the clot that forms is based almost exclusively on fibrin activity. The level of fibrinogen is estimated from the MA of the FF assay. The contribution of platelets to the clot strength can be directionally estimated from the difference in MA between the FF assay and the Kaolin assay, or through comparison with the RapidTEG™. The FF assay is usually performed using citrated whole blood.

PlateletMapping® Assays

TEG® PlateletMapping® assay for platelet function testing is performed using four separate assays as follows: (1) Kaolin assay (see above for details); (2) ActivatorF assay (includes heparin, reptilase, and factor XIIIa, resulting in a thrombin-independent, fibrin-based clot); (3) Arachidonic acid (AA) plus ActivatorF assay (generates a thrombin-independent, fibrin-based clot with additional contribution from platelets based on activation of the AA pathway); (4) Adenosine diphosphate (ADP) plus ActivatorF assay (generates a thrombin-independent, fibrin-based clot with additional contribution from platelets based on activation of the ADP pathway). PlateletMapping can be used to measure the effects of antiplatelet medication (e.g., ticagrelor, clopidogrel) or aspirin or to assess platelet function after a patient has experienced an injury [10].

Comparison with Standard Coagulation Tests

Standard laboratory tests of coagulation include activated partial thromboplastin time (aPTT), prothrombin time (PT), international normalized ratio (INR), and thrombin time (TT). In addition, levels of key blood constituents can be measured in the laboratory (e.g., platelets, red blood cells, Polymorphonuclear (cells) (PMNs), and coagulation factors such as fibrinogen). Figure 19.3 gives an overview of the in vitro coagulation process as measured through conventional coagulation tests.

The method of assessment with thromboelastography differs markedly from the methods of standard laboratory tests. Using whole blood, thromboelastography provides a range of results that characterize the entire coagulation process, from initial clot formation to lysis [1]. By including blood constituents such as platelets and red blood cells, the conditions for coagulation in the TEG® analyzer reflect those found *in vivo*. In contrast, standard laboratory tests are performed using platelet-poor plasma and they are stopped when the first fibrin strands are formed, meaning that they only assess the beginning of clot formation. Also, viscoelastic properties of the blood clot are not measured in any of the standard coagulation tests. These fundamental differences mean that no thromboelastography measurement should be considered as equivalent to a standard laboratory-based coagulation test. Instead, the two sources of data should be considered as complementary. However, some correlations have been observed, such as between FF-MA and the plasma fibrinogen level [5, 12].

Unlike most standard coagulation tests, thromboelastography can be undertaken at the site-of-care. In addition, only minimal training is needed in order to use the current TEG® 6s device [13] meaning that there is no need to rely on specific staff with extensive technical expertise. Moreover, early results from TEG® assays are available within ~15 minutes. Therefore, the turnaround time for obtaining TEG® results can be kept short, and it is generally accepted that TEG® is likely to enable treatment decisions to be

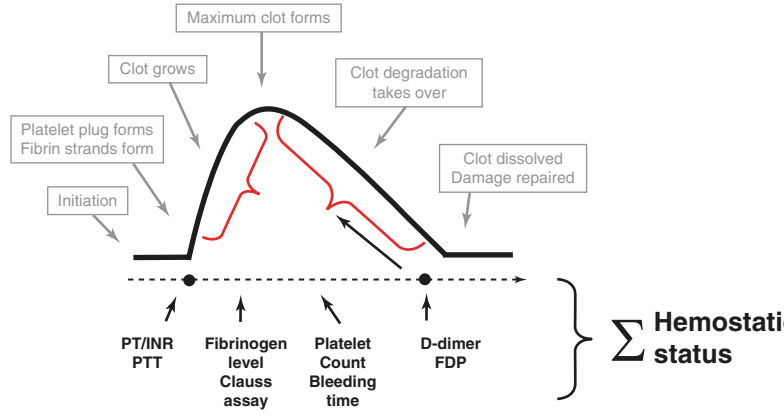


Fig. 19.3 The role of conventional coagulation tests in measuring hemostatic status. (Figure adapted from Agarwal et al. [11]). CCT conventional coagulation test, FDP fibrin degradation product, INR international normalized ratio, PT prothrombin time, PTT partial thromboplastin time

made earlier than with standard coagulation tests [14–16]. Furthermore, TEG® testing provides indications for specific blood components. This may be a crucial benefit, for example, when a patient has life-threatening bleeding, and there is an urgent need to understand what treatment is needed to achieve hemostasis.

Limitations

Variability between users has historically been an important limitation of thromboelastography testing [1, 17]. The main reasons for such variability were related to the methods for using the TEG® 5000, such as the need for manual pipetting and the requirements for calibration. The extent of variability is greatly reduced with the TEG® 6s device because the analysis procedure with this device is almost fully automated [9].

Another limitation of TEG technology is that the *in vivo* clotting conditions are not fully represented. The biology and physiology of the blood vessel are not replicated. Endothelial cells are believed to influence the coagulation process *in vivo*, but are not currently represented in thromboelastography. In addition, the physical effects of blood flow are not accurately reflected, particularly with the TEG® 6s methodology, and it may also be argued that the rotation of TEG® 5000 device does not accurately represent blood flow. Additionally, thromboelastography is limited in its ability to accurately detect certain deficiencies, such as von Willebrand disease.

Clinical Use

Thromboelastography in Trauma

The diagnostic utility of TEG® viscoelastic testing in the care of trauma patients has been extensively described in original research as well as in numerous recent review articles [18–24]. Many of the studies demonstrate the early diagnostic value of testing with TEG® technology in informing the right therapeutic strategy as well as in predicting short-term (e.g., need for massive transfusion) and midterm outcomes (e.g., mortality) [25–30]. Many studies suggest a higher diagnostic value of TEG® testing compared to standard coagulation tests (aPTT and PT/INR), and not just because of the faster turnaround of results [31–33]. Some have suggested that at admission TEG® testing can replace standard coagulation test in the trauma setting [14]. Multiple studies have used the TEG® technology to show hyper- or hypocoagulability in certain patient populations, for example, male versus female [34].

Multiple clinical trials have demonstrated the clinical benefits of TEG® testing in the treatment of trauma-induced coagulopathy, as well as of TEG®-guided resuscitation and massive transfusion protocols. Benefits of TEG® testing include reduced mortality and improved blood product and other resource utilization [35–39]. Multiple groups from Europe and the United States have developed and published TEG®-based transfusion algorithms for use in trauma [20, 40, 41].

A pragmatic, randomized clinical trial was performed to compare the outcomes in trauma patients treated using the TEG®-based algorithm shown in Fig. 19.4 versus an equivalent algorithm with guidance from conventional coagulation tests [42]. The study included 111 patients with a median injury severity score (ISS) of 30. Patients treated with the TEG®-guided algorithm showed significantly reduced mortality compared with those in whom conventional tests were used: 20% versus 36% ($p = 0.049$). The quantities of plasma and platelet administered during the first 2 hours after injury were higher among patients treated according to conventional tests, and the authors suggested that TEG® guidance facilitated the administration of appropriate treatment at an earlier time.

Another benefit of thromboelastography is the ability to characterize different stages or subtypes of trauma-induced coagulopathy, which allows for a more tailored, individual approach to the treatment of each patient. Using principal component analysis methodology, multiple groups were able to establish different phenotypes of trauma-induced coagulopathies; these include global coagulopathies with depletion of platelets

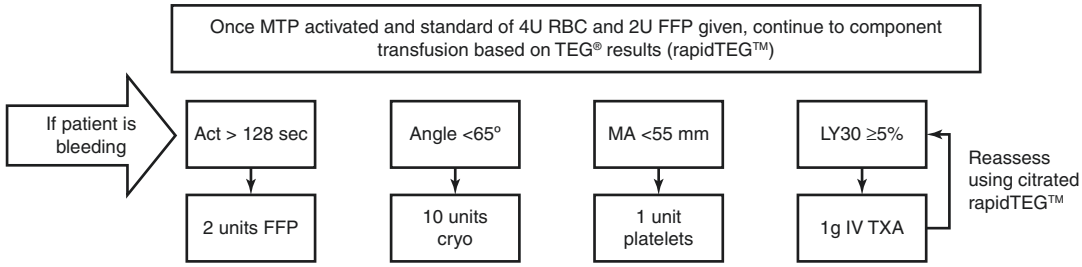


Fig. 19.4 TEG®-based algorithm developed for the treatment of trauma by massive transfusion protocol, modified after Gonzales et al. [42]

and fibrinogen or depletion of clotting factors, as well as (hyper-)fibrinolysis with or without activation of protein C [43–45].

Several studies have suggested that fibrinolysis plays an important role, particularly in high bleeding and high mortality trauma cases [27, 46–48]. As a result, the treatment of fibrinolysis with antifibrinolytic medications, such as tranexamic acid (TXA), has been suggested. In the CRASH-2 trial, a 1.5% mortality benefit was observed when TXA was administered to all trauma patients [49]. However, there were several shortcomings in the design of this trial [50], and questions were raised if antifibrinolytic therapy should only be given to patients who have confirmed increased fibrinolysis.

Viscoelastic tests are a reliable method to rapidly detect hyperfibrinolysis and the TEG® 6s Hemostasis Analyzer is (as of June 2020) the only FDA-cleared cartridge-based test that can detect hyperfibrinolysis in a trauma setting at the site-of-care. The FDA clearance was granted based on a clinical method comparison study with the TEG® 5000 analyzer that enrolled nearly 500 trauma patients [9, 51].

Hyperfibrinolysis identified by the TEG® LY30 parameter has been shown by several studies to be associated with increased mortality, increased need for massive transfusion and increased use of overall resource utilization [47, 52–54], suggesting goal-directed treatment with antifibrinolytic medications, for example, in patients with LY30 > 3% [8]. Conversely, patients without hyperfibrinolysis or with fibrinolytic shutdown do not seem to get a benefit from antifibrinolytics, or could even be harmed [55–57].

Other Clinical Applications

Liver Transplantation

Liver transplantation was among one of the first broadly adopted clinical areas of use of thromboelastography, beginning in the early 1960s [58]. Starzl et al. at the University of Colorado first suggested the selective use of antifibrinolytics based on an increased risk of pulmonary embolism. The combination of patients with severe underlying coagulopathy [59], paired with an intervention exposing them to a very high bleeding risk, created a significant unmet clinical need for holistic and timely hemostatic information.

Kang and his team in Pittsburgh further established the use of thromboelastography in liver transplantation and demonstrated the ability to identify specific coagulation disorders with thromboelastography that developed through the course of the procedure. They also demonstrated a reduction in transfusion volume by a third when using TEG® testing and published seminal papers on the role of hyperfibrinolysis as detected by thromboelastography [6, 60].

Today, thromboelastography remains a key component of intra- and perioperative hemostasis management in liver transplant patients, particularly for the assessment of coagulation factor deficiencies, platelet disorders, hyperfibrinolysis, and hypofibrinogenemia [61–65]. In addition, valuable information can be derived to differentiate heparin-induced bleeding from other intrinsic sources of bleeding [66]. Multiple algorithms have been proposed to optimize the workflow and utility of TEG technology, and to improve blood

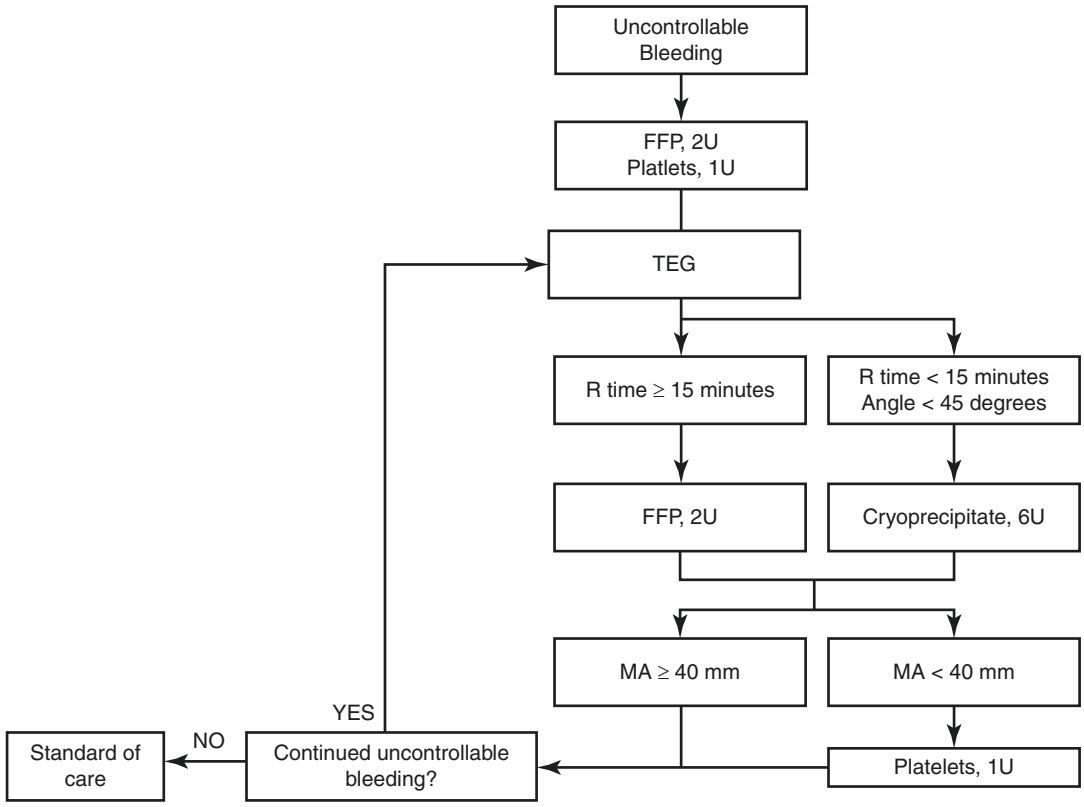


Fig. 19.5 TEG®-based algorithm developed for the treatment of bleeding during liver transplantation surgery, modified after Wang et al. [67, 68]

component utilization in cirrhotic patients; an example algorithm is shown in Fig. 19.5 [67].

Cardiac Surgery

The utility of thromboelastography with the TEG® analyzer, ranges from presurgery assessment, throughout the surgery itself (coronary artery bypass grafting [CABG] is the most frequent procedure) and eventually to the postoperative period. At each stage, thromboelastography can provide critically important information to improve the individual care for the patient and to use resources more effectively [69, 70].

In the preoperative assessment, thromboelastography can provide useful insight into the coagulation status of the patient. Many CABG patients are on antiplatelet therapy (e.g., P2Y12 receptor blockers, aspirin) and some may also take anticoagulants, such as warfarin or more recently direct-acting oral anticoagulants (DOACs; either direct thrombin inhibitors or Factor Xa inhibitors). It is therefore important to test for any residual drug effects [71]. Furthermore, when faced with a dilemma between timely intervention and remaining bleeding risk, for instance, in less critical or time-sensitive emergency cases, thromboelastography has been used to provide guidance and weigh the benefit/risk ratio. In doing so, damage caused by early interventions with high bleeding results can be prevented, and it can also help patients receive definitive care sooner, and reduce the overall length of stay [72–74]. Thromboelastography has been shown to identify manifest bleeding disorders preoperatively, and has good negative predictive value for intra- and postoperative bleeding [75].

Surgery requiring cardiovascular bypass (such as CABG and valve repair) can cause dilutional coagulopathy (similar to trauma). Moreover, the extensive contact with artificial surfaces (pump,

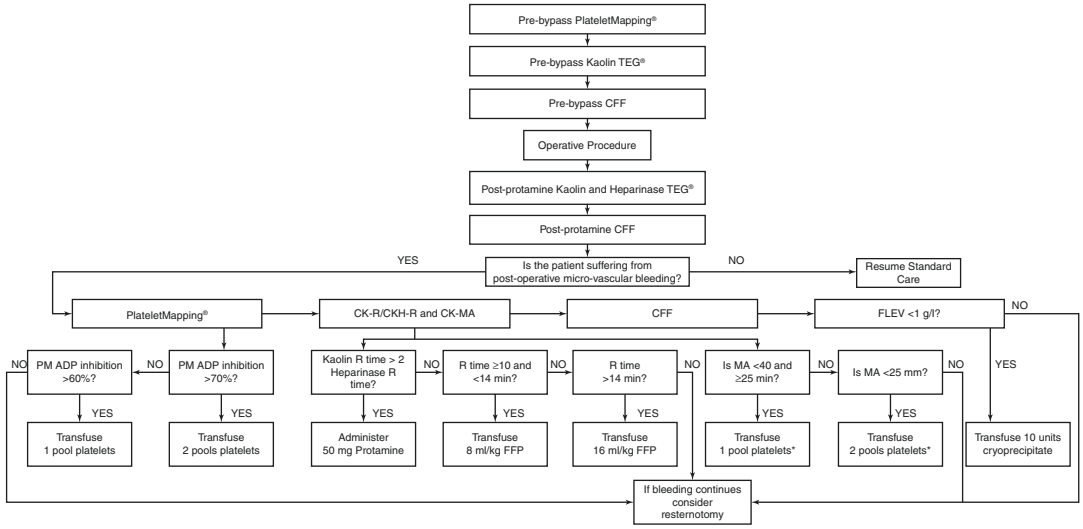


Fig. 19.6 TEG®-based treatment algorithm developed for use in patients undergoing coronary artery bypass surgery modified after Agarwal et al. [71]. *If not already receiving platelet transfusion from MEA/PM; ADP adenosine diphosphate, CFF citrated functional fibrinogen, FLEV level of fibrinogen in the plasma, MA maximum amplitude, R-time reaction time

etc.) can lead to aggravating consumptive coagulopathy. Thromboelastography has been used extensively to monitor the coagulation status of these patients. In some institutions, a specific TEG® test, the heparinase test, is performed to counteract the heparin effect observed during cardiovascular bypass surgery, and to allow the physician to "see through" and to assess the underlying coagulation status of the patient. Protamine is administered to reverse the heparin effect after full rewarming. This is a critical time as it is during this period that any coagulopathies would manifest further. It is also helpful at this stage to distinguish inherent from surgical bleeding [70, 73, 76].

In the postoperative period, thromboelastography remains a key diagnostic tool to assess the coagulation status of the patient and to manage hemostasis. Particularly, in cases of unexplained oozing or other manifest bleeding, thromboelastography can provide valuable information to get to the root cause of the bleeding, and can help inform the decision to re-explore a patient surgically [77].

Since the pioneering work in cardiovascular surgery in the 1980s and 1990s, multiple updated treatment algorithms have been proposed [70, 78–83]. One example, developed in the United Kingdom (Fig. 19.6), included the use of platelet function testing (both multiple electrode aggregometry and PlateletMapping®) before implementing cardiopulmonary bypass surgery, to determine the patient's anticipated post-bypass requirement for platelet transfusions [71]. A recent meta-analysis summarized the clinical benefits of using TEG® technology to guide bleeding and hemostasis management in patients undergoing cardiovascular surgery [75].

Obstetrics

As in trauma, hemorrhage is a major contributor to mortality in the obstetric setting, and an accurate holistic assessment of the coagulation status with thromboelastography provides additional diagnostic insight and can inform an individually optimized hemostatic therapy [84–86]. Given the general changes to the coagulation system during pregnancy, it is important to identify specific trigger points and to develop targeted algorithms for hemostatic intervention [87, 88]. Particularly for postpartum hemorrhage, thromboelastography has been recommended to be included in treatment algorithms [88–91]. Figure 19.7 shows an example algorithm for the use of thromboelastography in obstetrics [92].

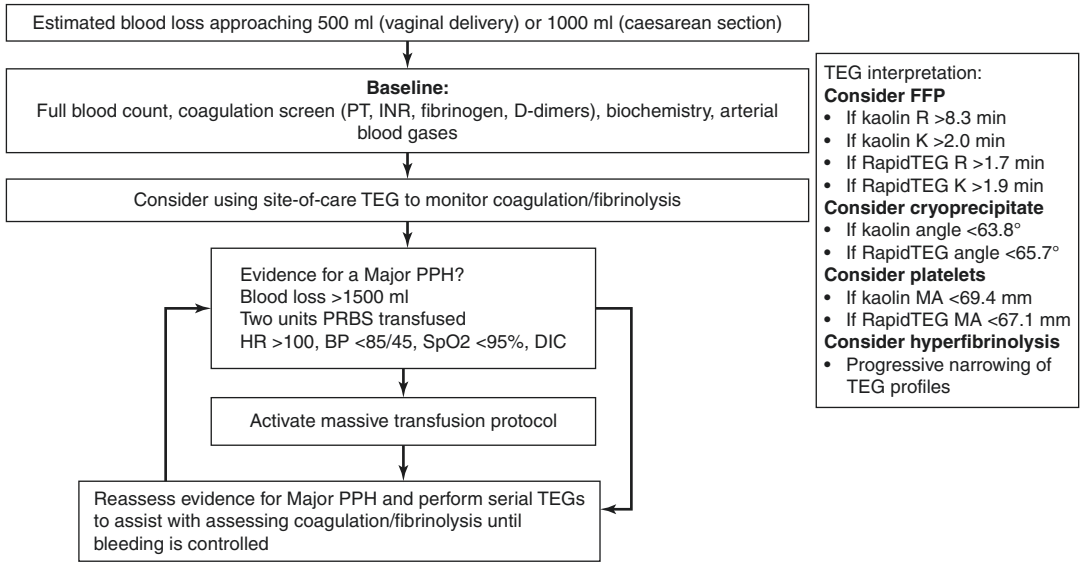


Fig. 19.7 TEG®-based treatment algorithm developed for use in obstetrics, modified after Hill et al. [92]

Interventional Cardiology

While the immediate bleeding risk for patients undergoing percutaneous coronary intervention (PCI) has been significantly reduced, the risk of bleeding due to post-interventional antiplatelet therapy (and sometimes anticoagulation), as well as the increased thrombotic risk (e.g., restenosis), remain. Thromboelastography not only offers a unique holistic view including information on platelet function (similar to other platelet function tests, such as VerifyNow® or Multiplate®) but also on general coagulation pattern [93].

Platelet function testing is essential to assess the patient's risk profile [74, 93], and thromboelastography has been shown in various settings to be a valuable and reliable test for this purpose [10, 94–96]. Consequently, thromboelastography has been used in various studies to successfully risk stratify patients or to even predict their risk for an event [94, 97, 98]. With the clearance of the cartridge-based TEG® 6s Hemostasis Analyzer, a lot of these tests can now be performed at the site-of-care. The new generation device has been validated in the cardiology setting by multiple teams [99, 100]. With the new guidelines recommending guided de-escalation therapy after percutaneous coronary intervention (PCI) based on the TROPICAL-ACS trial [101], platelet testing with the TEG® analyzer has gained renewed momentum. Other areas that could be explored are the use of TEG for patients undergoing transcatheter aortic valve repair/replacement (TAVR) [102, 103] or left atrial appendage closure (LAAC), e.g., with a Watchman® procedure.

Stroke

In stroke patients, thromboelastography can provide a holistic view of the coagulation status of the patient providing important clinical insights regarding acute and long-term bleeding or thrombotic risks. In patients with intracranial hemorrhage, resulting hypercoagulability has been described as measured by shortened R time and increased MA in the thromboelastography assay [104]. Likewise, studies with thromboelastography in acute ischemic stroke patients showed faster clotting as measured by a significantly reduced R time [105]. Thromboelastography has also been suggested as a potential individualized diagnostic for targeted secondary stroke prevention [106].

Congenital Bleeding Disorders

Thromboelastography has been successfully introduced into the clinical management of patients with congenital bleeding disorders,

particularly for hemophilia patients [107]. Thromboelastography has been used to capture and describe the phenotypical heterogeneity of individual patients, and in some cases to predict the varying clinical outcomes in patients with otherwise comparable standard coagulation tests or factor level results. Moreover, thromboelastography is used for guiding of routine prophylaxis as well as for determining the response to bypassing agents [108]. Some studies have used thromboelastography in perioperative bleeding management for hemophilia patients [109, 110]. With new therapeutic options like emicizumab (Hemlibra®, Roche) available, in which standard coagulation tests do not render reliable or actionable results, thromboelastography has been proposed as an alternative for effective monitoring [111].

DOAC Detection

The increasing use of DOACs, such as Factor Xa inhibitors (rivaroxaban, apixaban, edoxaban, and betrixaban) and direct thrombin inhibitors (dabigatran), as well as the availability of reversal agents for both classes of DOACs (andexanet alfa, idarucizumab), has increased the need to reliably detect and classify DOACs and to quantify their therapeutic effect and demonstrate the effect of reversal agents. Factor Xa chromogenic anti-Xa assays are reliable laboratory test, but they take a long time, and do not provide a functional readout. Furthermore, the chromogenic assay may not be available in many hospitals. A point-of-care urine test (DOAC dipstick) is available commercially outside the United States and has its own limitations. To date, the use of point-of-care viscoelastic testing has not been validated sufficiently in terms of sensitivity to relevant plasma levels of DOACs. However, recent developments of TEG® testing in this setting have shown promising results [112–114]. For example, a recent multicenter proof of concept trial has shown excellent results for DOAC detection and classification in an experimental setting using a modified TEG® 6s cartridge containing ecarin and human factor Xa as activators [115].

Site-of-Care Use

The TEG® 5000 analyzer is sensitive to vibration, not easily portable, and requires multiple pipetting steps to perform each assay. This has limited its use at the site-of-care significantly. While some hospitals have placed TEG® 5000 analyzers close to the emergency department (ED) or operating room (OR), the majority of hospitals have it in the central lab, preventing site-of-care use. The next generation, cartridge-based TEG® 6s Hemostasis Analyzer is much less sensitive to vibration and conveniently portable, opening up opportunities for use in site-of-care settings. These include remote areas and austere settings in the military context as well as uses during land (ambulance, evacuation, and mobile stroke unit) or air (helicopter, airplane) transport [116–118].

Guidelines

The use of thromboelastography is recommended in the guidelines of several scientific societies. Testing with TEG® Hemostasis Analyzers is recommended in trauma guidelines for assessing coagulopathy and guiding hemostatic treatment. Viscoelastic monitoring is also recommended in guidelines for the monitoring and managing of hemostasis in cardiac surgery, for platelet function testing in high-risk patients undergoing percutaneous coronary intervention, in liver transplant surgery, and in postpartum hemorrhage. Guidelines are summarized in Table 19.4.

Thromboelastography has demonstrated broad clinical, diagnostic utility across a variety of clinical indications. The introduction of a newer, more user-friendly device (TEG® 6s Hemostasis Analyzer) will likely support further adoption of this technology.

Table 19.4 Guidelines recommending the use of TEG® technology or viscoelastic monitoring in different clinical settings

Clinical area	Society/Organization/Institution	Recommendations and references
Trauma	Task Force for Advanced Bleeding Care in Trauma	European guideline on management of major bleeding and coagulopathy following trauma (2016): [119] Early and repeated coagulation monitoring using traditional coagulation tests (Grade 1A) and/or viscoelastic methods (Grade 1C) Treatment with fibrinogen concentrate or cryoprecipitate if significant bleeding is accompanied by viscoelastic signs of a functional fibrinogen deficit or a plasma fibrinogen level of less than 1.5–2.0 g/L (Grade 1C) Repeat doses of fibrinogen must be guided by viscoelastic monitoring and laboratory assessment of fibrinogen levels (Grade 2C)
	American College of Surgeons (ACS) Trauma Quality Improvement Program (TQIP)	The ACS Massive Transfusions in Trauma Guidelines: [120] POC-based transfusion protocol once major bleeding has been controlled TEG cut-off values for ICU transfusion of plasma, cryoprecipitate, fibrinogen concentrate, platelets, and antifibrinolytics
	SFAR (Société Française d'Anesthésie et de Réanimation)	The French Working Group on Perioperative Haemostasis: [121] Viscoelastic tests should be used to indicate hemostatic treatment and to make clinical staff more aware of the severity of trauma
Cardiac surgery	NICE (National Institute for Health and Care Excellence)	TEG® systems are recommended to help monitor blood clotting during and after heart surgery [122]
	ESA (European Society for Anaesthesiology)	Recommend the use of standardized VHA-guided hemostatic algorithms with predefined intervention triggers [123]
	American Society for Anesthesiologists	If coagulopathy is suspected (intra- or postoperatively), obtain standard coagulation tests or VHAs (e.g., TEG® and ROTEM® analyzers), if available [124]
	National Blood Authority Australia	In adult patients undergoing cardiac surgery, use of thromboelastography should be considered [125]
	SFAR (Société Française d'Anesthésie et de Réanimation)	The French Working Group on Perioperative Haemostasis: [121] VHAs should be used in the event of hemorrhage at the end of surgery and postoperatively VHAs should be included in algorithms
	EACTS (European Association for Cardio-Thoracic Surgery) and EACTA (European Association for Cardiothoracic Anaesthesiology)	Perioperative treatment algorithms for the bleeding patient based on viscoelastic POC tests should be considered to reduce the number of transfusions [126]
Platelet function testing	American College of Cardiology and American Heart Association and SCAI (The Society for Cardiovascular Angiography and Interventions)	Guideline for Percutaneous Coronary Intervention: Platelet function testing may be considered in patients at high risk for poor clinical outcomes [127]
	European Society of Cardiology and EACTS (European Association for Cardio-Thoracic Surgery)	Guidelines on Myocardial Revascularisation: [128] Platelet function testing may be considered to monitor antiplatelet therapy in specific high-risk situations (e.g., history of stent thrombosis; compliance issue; suspicion of resistance; high bleeding risk)

(continued)

Table 19.4 (continued)

Clinical area	Society/Organization/Institution	Recommendations and references
Liver transplant surgery	SFAR (Société Française d'Anesthésie et de Réanimation)	The French Working Group on Perioperative Haemostasis on viscoelastic tests: [121] Viscoelastic tests can be an aid in liver transplantation by limiting the transfusion of labile blood products
	ESA (European Society for Anaesthesiology)	Guidelines for the management of severe perioperative bleeding: [123] In visceral and transplant surgery, tranexamic acid is recommended for the treatment of fibrinolysis (evident from microvascular oozing or viscoelastic hemostasis assay clot lysis measurement) but not for routine prophylaxis
Postpartum hemorrhage	ESA (European Society for Anaesthesiology)	Viscoelastic hemostasis assays recommended to identify obstetric coagulopathy In severe PPH, a VHA-guided intervention protocol is recommended [123]
	The Association of Anaesthetists of Great Britain & Ireland	There should be equipment to enable bedside estimation of coagulation such as TEG® or ROTEM® analyzers [129]
	ISTH SSC (International Society on Thrombosis and Hemostasis Scientific and Standardization Committee)	Recommend monitoring hemostasis with either PT/aPTT and Clauss fibrinogen or POCTs using thromboelastometry during PPH [89] If TEM is used, management should be based on a local algorithm and a quality control protocol agreed with hematology
	SFAR (Société Française d'Anesthésie et de Réanimation)	The French Working Group on Perioperative Haemostasis on viscoelastic tests: [121] Fibrinogen concentration should be rapidly evaluated in the event of PPH and viscoelastic tests may be useful in this regard

aPTT activated partial thromboplastin time, *POCT* point-of-care test, *PPH* postpartum hemorrhage, *PT* prothrombin time, *VHA* viscoelastic hemostatic assays

TEG® Hemostasis Analyzer Operator tracings and images used by permission of Haemonetics Corporation

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Part V

Management of Trauma Induced Coagulopathy

Red Blood Cell Transfusion

20

Anne M. Winkler

Blood Collection and Transfusion-Service Related Activities

Blood Collection and Manufacturing

Transfusion medicine is a highly regulated discipline. In the United States (US), the Food and Drug Administration (FDA) provides oversight for blood and blood components to ensure protection of transfusion recipients [1]. Throughout this chapter, references are made to the Circular of Information for the Use of Human Blood and Blood Components, a publication of the AABB (formerly the American Association of Blood Banks), the American Red Cross, America's Blood Centers, and the Armed Services Blood Program; this Circular was designed as an extension of container labeling to provide specific instructions for the administration and use of blood and blood components intended for transfusion and can be a useful guide to prescribing clinicians [2].

In the US, blood donation, distribution, and transfusion services operate within a network of community and hospital-based blood collection centers, and transfusing facilities, which provide red blood cells (RBC), platelets (PLT), and plasma, derived from whole blood or apheresis donation. Allogeneic blood donation is voluntary, nonremunerated and donor screening includes a focused physical examination, hemoglobin (Hb) or hematocrit measurement, completion of a questionnaire about specific risk behaviors, travel, medications, and other factors that could affect the transfusion recipient or donor safety. Following successful eligibility and consent, whole blood is collected through a large gauge needle by gravity flow from the donor's antecubital fossa and into the primary bag of a sterile, disposable, plastic bag set, containing an anticoagulant-preservative solution to prevent clotting. After collection, whole blood is separated into individual components by centrifugation and further processed into RBC, plasma, and PLT destined to become pooled units. In comparison, apheresis blood collection uses specialized bag sets and automated instruments that are designed to continuously draw and centrifuge small volumes of blood, remove the desired component(s) (e.g., RBC, plasma, single donor PLT, and granulocytes), and return the remainder, with minimal impact to the donor's fluid balance. Other types of blood donation include those collected for exceptional medical need, directed, and autologous donation.

The volume of an allogeneic whole blood collection ranges from 450 mL (±10%) to 500 mL (±10%), depending on the collection system used [2]. RBC are collected into anticoagulant-preservative solutions contain-

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ing the following solution combinations: citrate–phosphate–dextrose (CPD), citrate–phosphate–dextrose–dextrose (CP2D), and citrate–phosphate–dextrose–adenine (CPDA-1) [2]. Citrate chelates ionized calcium in the donor's blood to inhibit coagulation. Phosphate and dextrose directly provide nutrients to the red cells. Adenine is a nucleic building block that is added to some RBC solutions (CPDA-1) and additive solutions (AS), allowing the RBC shelf life to exceed 3 weeks [2]. RBC collected in CPD and CP2D have a shelf life of 21 days, and those collected in CDPA-1 have a shelf life of 35 days. The volumes of RBC units vary between 225 and 350 mL with a hematocrit ranging from 65% to 80% [2].

AS contain combinations of phosphate, adenine, mannitol, dextrose (glucose), and additional citrate to provide nutrients and stabilize RBC membranes, allowing for increased storage times. As a result, AS (e.g., AS-1, AS-3, AS-5, and AS-7) are commonly added to the RBC unit to enable extension of the RBC shelf life to 42 days. These solutions add an additional 100 or 110 mL of fluid postcollection, which reduces the hematocrit to 55–60% with an increased volume of approximately 300–400 mL [2].

All donors are tested for blood type, RBC antibodies, and infectious disease markers; PLT are also tested for bacterial contamination before they are released [3]. The most recent National Blood Collection Utilization and Survey (NBCUS) reports that 12,591,000 whole blood and apheresis RBC units were collected in the US in 2015 [3]. Of those, 11,349,000 RBC units were transfused at US acute care hospitals, constituting a 13.9% decline since 2013 [3]. Nonetheless, the US continues to transfuse RBC at a greater rate than many other countries, with 35.3 transfusions per 1000 population [3].

ABO/Rh and Compatibility

The ABO blood group system was identified in 1900 by Landsteiner and colleagues and remains one of the most important medical discoveries, as prior to this time, there were deaths due to transfusion incompatibility [4, 5].

Codominant Mendelian inheritance of an A or B allele on chromosome 9q34 predicts blood type [6]. The A and B alleles each encode a glycosyltransferase which adds a sugar to the H antigen (*FUT1*, chromosome 19q13.3), an oligosaccharide chain that extends beyond the RBC surface [7]. Addition of a specific sugar, *N*-acetylgalactosamine or α-1, 3-galactose, results in the formation of an A or B antigen, respectively [7]. Type O results from homozygous inheritance of a nonfunctional allele most commonly caused by a frameshift mutation, resulting in no glycosyltransferase being produced, thereby leaving the H antigen unaltered [7].

Type A red cells express A surface antigen, and naturally occurring anti-B is found in plasma [8]. Conversely, type B red cells express B antigen and anti-A is present in plasma. Type O red cells lack A or B antigens and have anti-A and anti-B in the plasma. Finally, type AB red cells express both A and B antigens and lack naturally occurring anti-A and anti-B [8]. Naturally occurring blood group antibodies, also called isohemagglutinins, are not present in the newborn (apart from antibodies present due to passive maternal transmission) but develop around 4–6 months of age by a thymus-independent mechanism following exposure to carbohydrate epitopes on gut bacteria and food [8]. A and B antigens are also found on cardiac, gut, and renal endothelium (and other organs) and exist in a soluble form in secretions; for this reason, they are also known as "histo-blood group antigens" and as such are an important consideration in solid organ transplant [7] (Table 20.1).

ABO compatibility is fundamental to avoid a hemolytic transfusion reaction which may occur within minutes of the start of an RBC transfusion and possibly with fatal results. ABO antibodies are primarily IgM, which fix complement well, and can cause acute, intravascular hemolysis. ABO antibodies of the IgG subtype may cause a delayed, extravascular hemolysis. IgG subtypes may also cross the placenta from the maternal circulation to cause hemolytic disease

Table 20.1 Frequency of ABO and Rh (D) type and expected plasma antibodies [4, 9, 10]

ABO and RhD type by ethnicity and approximate percentages					Red cell antigen	Plasma antibody
	Caucasian	African–American	Hispanic	Asian		
O	44	49	55	43	None	Anti-A and Anti-B
A	43	27	28	27	A	Anti-B
B	9	20	13	25	B	Anti-A
AB	4	4	4	5	A and B	None
RhD Pos.	83	93	93	98		
RhD Neg.	17	7	7	2		

of the newborn (HDN) [11]. Only one fatality resulting from ABO-mismatched RBCs was reported to FDA in 2017, accounting for 3% of the total fatalities reported in that year [12]. Undoubtedly, many more ABO-mismatching events transpire without resulting in fatality. While the ABO blood group system is best known, there are 36 blood group systems currently recognized by the International Society of Blood Transfusion. Antibodies can develop to any of these blood group antigens, some of which have been implicated in hemolytic transfusion reactions and HDN. In 2017, six fatalities were attributed to non-ABO hemolytic transfusion reactions [12]. Variations or subtypes of common blood types are occasionally seen and may present challenges to the laboratory such that even routine blood orders require extra time to fulfill.

Pretransfusion Testing and Selection of RBC for Transfusion

Type, Screen, and Crossmatch

Persons with type O blood are often called the "universal donor" since their red cells are compatible with all recipients. Type O RBC is also the first choice of blood in emergency transfusion or trauma situations. Persons with A blood may receive type A or type O RBC; persons with type B blood may receive type B or type O RBC. Persons with type AB RBC may be given any blood type and are sometimes referred to as being the "universal recipient" (Table 20.2).

Table 20.2 Red cell type and compatibility

Recipient blood type	Compatible red cells for transfusion
O	O only
A	A or O
B	B or O
AB	O or A or B or AB

The Rh blood group system is composed of two genes that account for expression of 54 antigens. The D antigen is the most recognized Rh antigen and the presence or absence of the D antigen on the red cell is still commonly referred to as Rh-positive or Rh-negative, respectively. D antigen expression varies among ethnic groups (Table 20.1). With respect to transfusion, the D antigen is second in importance to the ABO blood group system. For routine RBC transfusion, every effort is made to match the ABO/Rh of the unit to the recipient. For example, a patient typing B-negative should ideally receive RBC from a donor who is B-negative (type B, RhD-negative), but O-negative RBC would also be compatible.

Anti-A and anti-B are naturally occurring antibodies and are present depending on blood type (Table 20.1). Non-ABO red cell antibodies are sometimes found in a patient's sample and are called "unexpected" alloantibodies. About 5% of patients have unexpected alloantibodies. These antibodies have formed following exposure to red cells possessing antigens foreign to the recipient usually from transfusion or pregnancy but possibly from other blood exposure. Antibodies differ in their clinical significance or in their ability to cause hemolysis and/or HDN. Extra time may be needed by the transfusion service to locate RBC for patients with rare or multiple alloantibodies.

If a "type and screen" is ordered, the "type" is the determination of the patient's ABO and RhD type and the "screen" detects unexpected alloantibodies in the patient's sample, such as anti-K (of the Kell blood group system), anti-Fya (of the Duffy blood group system) and so on. Multiple blood group systems are represented on screening and extended reagent red cell panels to improve the chances of detecting clinically significant alloantibodies. Once a person has formed an alloantibody, RBC negative for the offending antigen should be provided if possible, whether the alloantibody is detectable. If a "crossmatch" is ordered, this is the testing of patient plasma against the intended donor red cells and it is the last check of compatibility prior to issue. If there is agglutination or hemolysis, the unit is incompatible. Crossmatched RBC units may be reserved for a designated time, depending on the institution's policies. Emergency-release RBC are usually O-negative or O-positive and are not crossmatched. As a result, the release of these RBC units requires a physician's signature to approve the product, either prior to or in a specified timeframe following release. Uncrossmatched blood is not necessarily incompatible.

Selection of RBC for Transfusion

The first choice of RBC in a patient with an unknown blood type is O, since blood type O individuals possess no A or B red cell surface antigens and are therefore more likely to be compatible with any recipient. Upon receiving an alert of an incoming trauma or for emergency RBC needs, most hospitals automatically issue O RBC units, unless the patient's blood type is already known. In most emergency release protocols, women of childbearing potential (or age) receive O-negative RBC until their blood type is known. What constitutes childbearing age is determined by each institution but is generally accepted to be females under the age of 45 or 50. If a woman of childbearing age is confirmed as O-negative, she should continue to receive O-negative RBC to decrease her risk of forming anti-D that is capable of crossing the placenta which can result in HDN. Another group that may automatically receive O-negative RBC is patients under 18 years of age. For women beyond childbearing potential and all males greater than 18 years, O-positive RBC may be issued before pretransfusion testing is complete. This approach has been adopted in many large, urban institutions as the need for emergency release RBC could never be sustained if only O-negative RBC were used. Once the patient's blood type is known, type-specific or compatible RBC may be given.

RBC Transfusion Indications and Administration

Indications

RBC transfusion is used to increase oxygen carrying capacity in patients with anemia in whom physiologic compensation is inadequate to maintain tissue oxygenation. Patients may require RBC transfusion in situations including hemorrhagic shock, other blood loss such as that from surgery and symptomatic anemia. Signs and symptoms of anemia that may prompt RBC transfusion include hemodynamic instability, chest pain, shortness of breath, and tachycardia at rest. In nonbleeding patients, Hb levels are typically used to guide transfusion decisions. Unfortunately, Hb concentration alone is a poor measure of circulating RBC mass because of the physiologic compensatory mechanisms that preserve oxygen transport such as reduced blood viscosity to increase blood flow to tissues, redistribution of blood flow, increased unloading of oxygen to tissues, and maintenance of blood volume due to expansion of plasma volume. Because of this and observations made in Jehovah's Witness patients who decline transfusion based on religious beliefs and in underdeveloped countries where RBC were unavailable or limited, readjustment of transfusion practice to a lower Hb threshold has been investigated [13–18]. In 2012, a Cochrane systematic review of prospective randomized trials compared restrictive versus liberal transfusion strategies in 19 trials

including 6264 patients [19]. The authors found that a restrictive transfusion strategy reduced the risk of receiving an RBC transfusion by 39% without an increase in adverse events, intensive care unit or hospital length of stay, and 30-day mortality. The authors concluded that the existing evidence supported the use of restrictive transfusion triggers in most patients. For RBC transfusion, multiple prospective randomized trials have been conducted to investigate restrictive versus liberal transfusion thresholds in adult patients in critical care, cardiac surgery, hip fracture repair, acute upper gastrointestinal bleeding, and septic shock, and the results have been summarized in Table 20.3 The seminal Transfusion Requirements in Critical Care or TRICC trial was the first study to demonstrate that in critically ill, euvolemic patients, a restrictive RBC transfusion approach (Hb threshold of 7 g/dL and maintenance between 7 and 9 g/dL) was at least as effective and possibly superior to a liberal strategy (maintenance Hb concentration of 10–12 g/dL). In addition, the restrictive strategy (threshold 7 g/dL) resulted in a 54% decrease in RBC transfusions and a decline of 33% in RBC exposure. As a result of these findings, clinical practice guidelines have adopted recommendation of a restrictive transfusion strategy using a Hb threshold of 7–8 g/dL; however, these recommendations may not be safe for all patients including patients with acute coronary syndrome.

Transfusion Administration

Safe administration of a blood transfusion requires multidisciplinary collaboration between healthcare providers. Informed consent must be provided and signed prior to transfusion of any blood product, by the patient receiving the transfusion or by a legally authorized representative or surrogate depending on state and local laws. If no one is available to provide consent and the transfusion is considered a medical emergency, it can be administered based upon the doctrine of implied consent; however, requirements may vary, and the emergent need must be documented in the medical record. Before a transfusion commences, a "time-out" should be performed by staff administering the transfusion to ensure the right patient is being transfused the correct blood product. Every hospital should have policies, processes, procedures, and training in place for all personnel involved in administering a transfusion. Certain religious faiths may decline blood product transfusion and their refusal should be respectfully honored and carefully documented according to hospital policy and applicable laws.

Blood components must be administered through special blood infusion filter sets, which typically have 150–260 μm filters to trap clots and particulate aggregates, but still allow blood cells to pass through [2]. No medications other than 0.9% sodium chloride should be administered through the same tubing at the same time.

Routine transfusions should be administered slowly (approximately 2 mL/min), especially in the first 15 minutes, to observe for signs and symptoms of a transfusion reaction. Vital signs should be taken prior to the transfusion and then according to institutional policy. After the first 15 minutes, the rate of transfusion should be increased to ensure the unit is transfused within 4 hours. Rapid infusion, unless medically necessary, should be avoided to mitigate the risk for transfusion-associated circulatory overload (TACO) especially in recipients with cardiac and/or respiratory compromise.

If a transfusion reaction is suspected, the transfusion should be stopped, patency of the intravenous line maintained, and health care provider notified. In addition, the transfusion service should be notified as soon as possible.

Intraoperative Blood Salvage

Specialized devices or "cell savers" may be used during planned or emergency surgery in which blood loss is excessive (equal to or greater than 20% total blood volume), for patients with religious objections to receiving allogeneic transfusion or for patients with multiple alloantibodies or rare blood types.

Table 20.3 Key randomized, controlled clinical trials investigating restrictive versus liberal transfusion strategies in adults

Study	Number of subjects randomized	Study setting	Hb concentration threshold (restrictive vs liberal)	Average Hb concentration at transfusion (restrictive vs liberal)	Number of patients transfused (restrictive vs liberal)	Primary outcome
Hébert et al. [20]	838	Intensive care unit	7.0 g/dL vs 10.0 g/dL	8.5 g/dL vs 10.7 g/dL	67% vs 100%	30-day mortality was 18.7% in the restrictive group compared to 23.3% in the liberal group ($p = 0.11$) while in-hospital mortality was lower in the restrictive group (22.2% vs 28.1%, $p = 0.05$)
Hajjar et al. [21]	512	Elective cardiopulmonary bypass	8.0 g/dL vs 10.0 g/dL	9.1 g/dL vs 10.5 g/dL ($p < 0.001$)	47% vs 78% ($p < 0.001$)	Restrictive strategy was noninferior to liberal in the primary composite endpoint (30-day mortality, cardiogenic shock, ARDS, or acute renal injury require dialysis or hemofiltration) occurring in 11% versus 10%, respectively ($p = 0.85$)
Carson et al. [22]	2016	Primary surgical repair of a hip fracture with cardiovascular risk factors	8.0 g/dL vs 10.0 g/dL	7.9 g/dL vs 9.2 g/dL ($p < 0.001$)	41% vs 97% ($p < 0.001$)	Rates of death or inability to walk without human assistance at 60 days were similar in the restrictive versus liberal group, 34.7% versus 35.2% ($p = 0.90$)
Villaneuva et al. [23]	921	Acute upper gastrointestinal bleeding	7.0 g/dL vs 9.0 g/dL	7.3 g/dL vs 8.0 g/dL ($p < 0.001$)	49% vs 86% ($p < 0.001$)	45-day mortality was reduced in the restrictive group (5%) compared to the liberal group (9%, $p = 0.02$)
Holst et al. [24]	1005	Intensive care patients who fulfilled criteria for septic shock	7.0 g/dL vs 9.0 g/dL	Daily lowest Hb differed between groups[a] ($p < 0.001$)	64% vs 99% ($p < 0.001$)	43% of the restrictive group and 45% of the liberal group died at 90 days after randomization ($p = 0.44$)
Mazer et al. [25]	5035	Cardiac surgery with a EuroSCORE I of 6 or more	7.5 g/dL vs 9.5 g/dL in the operating room or intensive care unit or 8.5 g/dL in non-ICU ward	Postoperatively, Hb concentrations were separated by approximately 1 g/dL and remained separated from ICU admission through day 28[a]	52.3% vs 72.6% ($p < 0.001$)	Composite outcome (death from any cause, nonfatal myocardial infarction, stroke, or new-onset renal failure with dialysis, occurring during the index hospitalization from the start of surgery until either hospital discharge or 28 days after surgery, whichever occurred first event) was 11.4% in the restrictive-threshold group compared to 12.5% in the liberal-threshold group ($p < 0.001$ for noninferiority)

Hb hemoglobin, *ARDS* acute respiratory distress syndrome, *EuroSCORE* European System for Cardiac Operative Risk Evaluation, *MODS* multiple organ dysfunction syndrome

[a]Numerical means/medians not reported

Various commercially-available devices function in essentially the same manner: intraoperatively shed blood is gently suctioned to preserve red cell morphology and function, washed with isotonic solution, filtered, and reinfused either intraoperatively or postoperatively. Sponges used intraoperatively may also be washed and rinsed and that fluid can be added into the circuit. Abdominal, thoracotomy, and drain blood from other surgical sites may be processed. Extracorporeal anticoagulation is achieved with heparin (up to 30,000 units per liter of normal saline) or citrate solutions (often ACD-A) or a combination of the two. The filters used have fairly large pore sizes (40–120 μm) to remove debris such as bony spicules or cement and large cellular aggregates. Due to washing, very little plasma or its solutes (e.g., free Hb, interleukins, and coagulation factors) remain in the final product.

Depending on the processing, the final product may be kept at room temperature for up to 4 or 6 hours, or at 1–6 °C for up to 24 hours in a monitored refrigerator [26, 27]. Since the whole blood product is freshly obtained from the patient and washed, some transfusion reaction risks are reduced, but reactions may still occur, such as from fluid overload or bacterial contamination [28]. The final red cell product is of high quality, with a hematocrit between 40% and 80% depending on the device and method used and is suspended in a small amount of isotonic solution with very little remaining anticoagulant. It has been shown that red cell survival of cell saver units obtained during cardiopulmonary bypass is comparable to circulating venous blood after 24 hours [29].

The literature is not abundant regarding the consistent use of autologous salvaged blood or autotransfusion in the setting of trauma, specifically regarding the quality of shed hemothorax blood as most current autotransfusion data are obtained from scheduled cardiac surgeries. A recent prospective observational study of unwashed hemothorax shed blood from 62 subjects at a large trauma center found significantly elevated cytokine levels as compared to normal controls, suggesting the potential for deleterious effects from autotransfusion in trauma [30]. Moreover, a second study demonstrated plasma hypercoagulability and platelet dysfunction induced by hemothorax frozen plasma from 17 adult trauma patients [31]. As a result, more studies including randomized trials are needed to determine the safety and efficacy of autotransfusion in trauma patients.

RBC Product Modifications

Clinical indications for modified components vary and it may be advantageous to consult with the transfusion service prior to ordering these products. Not all products are readily available and considerable time may be required to manufacture or obtain these modified RBC units.

Leukocyte Reduction and Provision of CMV-Negative RBC

Leukocyte reduction or leukoreduction (LR) of RBC is widely performed. Prestorage LR is done during automated apheresis collections or after whole blood collection. Bedside LR or poststorage LR remains an alternate but infrequently used. The FDA requires the residual white cell count to be less than 5×10^6 per RBC unit. LR of RBC decreases the incidence of febrile nonhemolytic transfusion reactions, human leukocyte antigen (HLA) and platelet alloimmunization, and the amount of biologic response modifiers (BRM), which accumulate during storage [32–34].

The equivalency of LR to Cytomegalovirus (CMV)-negative products continues to be debated. Some physicians consider LR RBC to be adequate and essentially equivalent to LR RBC from cytomegalovirus CMV-seronegative donors. The rationale is that CMV resides within white cells, and with the efficiency of modern LR filters, any risk of CMV would be exceptionally low [35–37]. For CMV-seronegative patients, especially those who are peri- or post-transplant, or low-birth-weight infants, many clinicians request CMV-negative products and accept LR-only products if CMV-negative LR RBC are unavailable. In addition, CMV-seronegative donors who test “negative for CMV” on their

most recent donation by antibody testing carry a small but real risk of transmitting CMV to a recipient, if the donor happens to be newly infected and is in the window period (the time between infection and the time at which the infection can be detected by testing). Nucleic acid testing (NAT) testing for CMV DNA to reduce this window period is available but is not routinely used for donor screening. Indications for CMV-negative blood include low-birth-weight infants born to CMV-seronegative mothers and hematopoietic stem cell or solid organ transplant recipients [2, 38].

Washing

Washed RBC are indicated in cases of an IgA-deficient recipient or in the rare case of an anaphylactoid/anaphylactic transfusion reaction [2]. Washed cellular products may also be requested for pediatric patients with renal impairment, elevated potassium, and related issues, to remove excess potassium in the blood product [39, 40]. However, for the vast majority of patients, the amount of potassium in the plasma should not have any untoward effects on the recipient even if the RBC are irradiated and near the outdate, if infusions are given slowly, over 2–4 hours.

Frozen RBC must also be washed prior to infusion, to remove glycerol in which they are stored [2]. RBC are washed by specialized, automated instruments that progressively wash a single unit in normal saline, and sometimes dextrose, to create a final product that is essentially devoid of plasma and which contains a minimal amount of saline. A small percentage of the product is normally lost during the washing process [2]. Washing reduces the RBC expiration date to 24 hours or the original expiration date and time, whichever comes first [2]. As a result, it is important to communicate to the transfusion service, the time frame in which the product will be needed.

Irradiation

Cellular blood products may be irradiated to preclude the development of transfusion-associated graft-versus-host disease (TA-GVHD), which is donor T-cell-mediated destruction of the recipient's immune system. TA-GVHD is most commonly caused by infusion of competent donor T-lymphocytes into an immunocompromised recipient, though there have been cases involving immunocompetent recipients [41]. TA-GVHD is similar to post-transplant GVHD, affecting HLA antigen dense tissues such as the skin, gastrointestinal tract, and liver. There are, however, two findings seen with TA-GVHD: bone marrow aplasia and earlier onset, usually between day 2 and 50 following transfusion, which distinguishes it from post-transplant GVHD.

Irradiation of cellular blood products is accomplished by X-ray or gamma-ray irradiators specifically manufactured for blood establishments, or by linear accelerators used in the field of radiation oncology [42]. Whatever energy source is used, irradiation renders residual allogeneic T-lymphocytes incapable of replication by rendering the leukocyte DNA inactive to a level greater than 5 logs. Irradiation causes damage to the red cell membrane and escape of intracellular potassium, which increases with the age of the red cell and over storage, but is usually not harmful to the recipient [39, 40]. Red cells outdate at 28 days from the date of irradiation or keep the original expiration date, whichever comes first [2].

Irradiated RBC are indicated for use in patient groups at risk for TA-GVHD including intrauterine transfusion, recipients of a cellular blood component from a blood relative, HLA-matched products, patients who have received a bone marrow or hematopoietic stem cell transplant, or patients on nucleoside (purine) analogs or T-cell function altering drugs (e.g., fludarabine, clofarabine, and alemtuzumab) [2].

Adverse Events Related to Transfusion

Transfusion complications or transfusion reactions, may be broadly divided into infectious and noninfectious serious hazards of transfusion. To decrease transfusion-transmitted infection, the FDA requires donor screening, which includes testing for human immunodeficiency virus (HIV)

types 1 and 2, hepatitis B and C virus, human T-cell lymphotropic virus (HTLV) types I and II, *Treponema pallidum* (the organism that causes syphilis), West Nile Virus (WNV), and Zika Virus. Recently, the FDA called for regional testing for *Babesi microti* or pathogen reduction in Babesia-risk states. Donors must be negative for antibodies to the parasite *Trypanosoma cruzi*, which causes Chagas disease, once in their donation lifetime. In regard to other infectious diseases, the donor questionnaire is written in a way such that donors with the possible risk of transmitting diseases such as malaria or hepatitis A are deferred from donation. Testing for CMV and HLA antibodies may be additionally performed. Donors may be temporarily, indefinitely, or permanently deferred based on the criteria set by FDA or AABB [43].

Much of the infectious disease testing is antibody-based and detects the donor's immune response to the offending agent. Adequate time, days to weeks, must pass until antibodies form; this also known as the window period. NAT detects viral nucleic acid particles and has greatly reduced the window period to just days. For example, prior to NAT testing for HIV-1, the window period for detection of antibody formation, even with third-generation tests, was 21–24 days after infection; in contrast, NAT testing reduces the window period to less than 10 days [44, 45] (Table 20.4).

Table 20.4 US blood donor screening and risk of recipient infection [46–50]

Infectious agent	Risk of infection per number of transfused units
HBV	1:800,000 to 1:1,200,000
HCV	1:1,100,000
HIV	1:1,500,000
WNV	Very rare; two cases reported 2008–2014
HTLV	1:641,000
Chagas	Very low, no cases since screening has been implemented (FDA 2010)
Syphilis	Very low, no cases in the past 40 years
Bacterial contamination of platelets	1:3000

With current screening and testing measures and the relatively low risk of transfusion-transmitted infection, the focus has shifted to noninfectious serious hazards of transfusion. The rate of these adverse reactions, commonly termed transfusion reactions, has been reported as 660 per 100,000 individuals from an international registry [51]. More specifically, in the US, there were 239.5 adverse reactions reported per 100,000 units transfused. A summary of the prevalence, signs and symptoms, and management of transfusion adverse reactions is presented in Table 20.5. Transfusion-related acute lung injury (TRALI), and TACO are discussed in detail.

Transfusion-Related Acute Lung Injury

TRALI continues to be a significant and underreported cause of transfusion-related morbidity and mortality in the US. TRALI has previously been reported as occurring as frequently as 1 in 3000 to 1 in 5000 transfusions, but the true incidence of TRALI is unknown as transfusion reaction reporting is voluntary [53]. Critically ill patients have up to an 8% incidence of TRALI, and has been reported in as high as 15% in patients with gastrointestinal bleeding and 30% in patients with end-stage liver disease [54, 55]. While TRALI can be fatal, the vast majority of patients recover within 96 hours, with aggressive, supportive treatment [56]. Nine TRALI-related deaths were reported in the US in 2017 [12].

Post-transfusion reactions consistent with what would now be described as TRALI were first reported in the 1950s; however, the term "TRALI" was coined by Popovsky and Moore in the mid-1980s and the constellation of findings and symptoms temporally related to transfusion were unified under one diagnosis [57]. The diagnosis of TRALI begins with the recognition of acute lung injury (ALI), defined as $SpO_2 < 90\%$ or $PaO_2/FiO_2 > 300$ mmHg on room air or other demonstration of hypoxemia and bilateral pulmonary edema seen as lung infiltrates by frontal chest radiograph [58]. A combined definition of

Table 20.5 Adverse reactions to transfusion [52]

Reaction	Prevalence (per 100,000 units transfused)	Signs and symptoms	Management
Allergic transfusion reaction	112.2	Urticaria, rash, skin itching, and swelling (throat, eye, tongue, etc.)	Antihistamines
Anaphylactic transfusion reaction	8	Bronchospasm, dyspnea, angioedema, hypotension, and tachycardia	Epinephrine, corticosteroids, antihistamines, fluid bolus
Acute hemolytic transfusion reaction	2.5–7.0	Fever, chills, dyspnea, hypotension, tachycardia, back pain, nausea, vomiting, oliguria/anuria, hemoglobinuria, and positive direct antiglobulin test (DAT)	Symptomatic treatment, diuretics and fluid administration For future transfusion, antigen negative RBC will be provided
Delayed hemolytic transfusion reaction	40	Occurs 2–14 days after transfusion; jaundice, anemia, elevated billirubin, reticulocytosis, spherocytosis, increased lactate dehydrogenase, positive antibody screen, and positive DAT	Symptomatic treatment For future transfusion, antigen negative RBC will be provided
Delayed serologic transfusion reaction	48.9–75.7	Occurs 2–14 days after transfusion; positive antibody screen, and positive DAT	Symptomatic treatment For future transfusion, antigen negative RBC will be provided
Febrile nonhemolytic transfusion reaction	1000–3000	Occurs within 4 hours of transfusion; temperature of 100.4 °F (38 °C) or increase of 1.8 °F (1 °C) from pretransfusion value with or without chills and rigors	Antipyretic or close observation
Post-transfusion purpura	Unknown (varies by component)	Occurs 2–14 days after transfusion; severe thrombocytopenia, petechiae, purpura, and identification of platelet antibodies	Self-limiting, intravenous immunoglobulin with or without corticosteroids For future transfusion, antigen negative platelets will be provided
Septic transfusion reaction	0.03–3.3	Fever, chills, hypotension, tachycardia	Antipyretic, empiric, antibiotics Culture blood product
Transfusion-associated circulatory overload	10.9	Occurs within 2 hours of transfusion; dyspnea, tachycardia, hypertension, headache, and jugular venous distension	Diuretic administration, reduce fluid intake

TRALI, as defined by the National Heart, Lung and Blood Institute (NHLBI) Working Group and the Canadian Consensus Conference is an acute, noncardiogenic lung injury occurring within 6 hours of transfusion with respiratory symptoms of tachypnea, dyspnea, and pulmonary edema which may be mild to severe and sometimes seen as complete "white-out" on frontal chest X-ray [59]. Frothy secretions are sometimes seen coming from the patient's mouth or endotracheal tube. If measured, the pulmonary artery wedge pressure should be less than 18 mmHg and there must not be any new, abnormal cardiac function [59]. Per this definition, TRALI may also be diagnosed in a patient with worsening preexisting pulmonary insufficiency (unique to the NHLBI definition), such as chronic obstructive pulmonary disease or pulmonary fibrosis [59]. A diagnosis of possible TRALI may apply to patients with preexisting clinical risk factors for ALI, such as recent surgery, burn injury, coagulopathy, chronic alcoholism, sepsis, and carcinoma [59]. Hypotension, fever, chills, nonproductive cough, and transient decreases in

white cell counts, especially neutrophils may also occur [59–61].

TRALI is a diagnosis of exclusion and septic transfusion reaction, volume overload, severe anaphylaxis, or a newly manifesting problem are often in the differential. If TRALI is suspected during the infusion of multiple consecutive products, all products given within a 6hour time frame are implicated. All routinely transfused blood products (whole blood, RBC, PLT, plasma, and cryoprecipitate) have been implicated in cases of TRALI [53].

The pathophysiology of TRALI is attributed to factors present in both the recipient and the transfused blood product. Up to 85% of TRALI cases may be explained by the infusion of donor antibodies as demonstrated in an ex vivo animal model using isolated perfused rabbit lungs [62]. In these studies, antibodies against HLA or human neutrophil antigens (HNA) had the ability to bind to neutrophils which expressed the cognate antigen and induced pulmonary edema. However, the antibody, the cognate antigen on the leukocyte surface, and the source of complement had to be present in order for ALI to occur and if any component was omitted, lung damage was obviated [62]. This model has been refined and demonstrated; a minimum number of antigen sites are needed to be present on neutrophils such that antibody binding must reach a threshold before ALI occurs [63]. This work was also relevant in showing that priming with N-formyl-methionine-leucine-phenylalanine, a component of bacterial cell walls allowed anti-HNA antibodies to directly activate neutrophils in the absence of complement [63].

In vivo TRALI models have demonstrated that a specific monoclonal antibody could cause ALI at a concentration seemingly similar to relatively well patients who receive a transfusion and develop TRALI [64]. However, in an animal model, when mice were housed in a pathogen-free environment TRALI was not demonstrated indicating a likely two-event model [64, 65]. Such an in vivo two-event pathogenesis was confirmed in a rat model which also demonstrated that lipids and other BRM could cause TRALI in older, stored RBC irrespective of LR [66]. Both antibodies to major-histocompatibility-complex (MHC) class I and the lipids from stored RBC were capable of priming quiescent neutrophils (PMN), activating primed PMN, and inducing ALI [66]. Specifically, rats that were infused with endotoxin (lipopolysaccharide, or LPS) alone did not develop ALI; however, those rats that received LPS and then a lipid extraction from 42dayold RBC did develop ALI [66]. Importantly, extractions from fresh RBC or plasma did not result in ALI [66]. In addition, antibodies to common MHC class I or even class II antibodies caused ALI as the second event in this animal model of TRALI, indicating that TRALI, whether caused by antibodies to specific leukocyte antigens or due to BRM that directly prime PMN, appears to be the result of two distinct events [66]. The first event is the clinical condition of the patient, which predisposes to TRALI and the second event is the infusion of the specific antibody or BRM into the patient which activates the sequestered PMN inducing damage to the vascular endothelium, resulting in capillary leak and ALI [53, 66].

TRALI mitigation strategies are vital to reducing morbidity and mortality related to this transfusion complication. During the 1990s, the UK National Blood Service created a voluntary hemovigilance program and was the first to collect plasma from donors, after noting that TRALI or probable TRALI was seen almost seven times more frequently with plasma transfusion and about eight times more frequently with PLT transfusion when these products were collected from female donors [67]. Their efforts were rewarded following the adoption of this collection strategy for PLT and plasma, and reports of TRALI from plasma transfusion fell from 15.5 cases per million units transfused (1999–2004) to 3 per million (2005–2006) for plasma and from 14 cases per million to just under six cases per million for PLT products [67]. Densmore and colleagues also showed that HLA antibody formation increased with pregnancy, with about 8% of never-pregnant females showing sensitization, increasing to 15% after one pregnancy, and to about 26% after three pregnancies [68]. Never-transfused men and never-pregnant women have a 1.7% prevalence of HLA antibodies [69]. The American Red Cross also examined hemovigilance data and subse-

quently adopted a similar strategy of collecting plasma from predominantly male donors (95%) and saw a significant (80%) decrease in TRALI cases related to plasma infusion [70].

Effective April 2014, AABB Standards state that the collection of plasma and whole blood for allogeneic donation must be from males, never-pregnant females, or females with a history of pregnancy only if they have been tested for HLA antibodies since their last pregnancy and are found to be negative.

Additional mitigation strategies include the use of licensed, pooled, and solvent-detergent-treated plasma products (Octaplas™ Pooled Plasma (Human) Solvent/Detergent Treated; OctaPharma, Lachen, Switzerland). European hemovigilance systems have not yielded any TRALI cases after using these products for 10–20 years' time. This may be explained because the pooling of numerous units dilutes the concentration of antibody and by the presence of soluble HLA antigen which is able to bind free antibody.

Transfusion-Associated Circulatory Overload

TACO is an acute, hydrostatic pulmonary edema which occurs in the setting of transfusion. Especially vulnerable populations include the very young, older patients (over 70 years of age), and those with compromised vascular systems or renal failure. TACO closely followed TRALI in the number of FDA-reported transfusion-related deaths, with 11 confirmed cases in the fiscal year 2017, comprising 30% of transfusion-related deaths [12].

There are several distinguishing factors between TRALI and TACO (Table 20.6). Pulmonary wedge pressure is an invasive measurement to determine the backpressure from the heart, or filling ("wedge") pressure of the left atrium, by a catheter in the pulmonary artery, and unless the patient already has a Swan-Ganz catheter in place it is not likely to be performed. Brain natriuretic peptide (BNP) is a 32 amino acid polypeptide secreted in response to stretched cardiac ventricles, to counteract the renin–angiotensin–aldosterone system. Baseline BNP is not likely to be measured unless the patient is already being monitored for heart failure and may not be useful unless the differences are marked. In general, two of the most helpful distinguishing features are that TACO responds quickly to diuresis, whereas this approach should be avoided in TRALI unless the patient is also fluid overloaded, and, though fever may or may not be seen with TRALI, it is never a feature of TACO. It may be helpful to make the patient sit upright; oxygen should be given as needed. It is possible that TRALI and TACO may occur together.

Table 20.6 Some distinguishing features between TRALI and TACO [2, 71]

	TRALI	TACO
Pulmonary edema, bilateral	Yes	Yes
Fever	Possibly	No
Tachypnea, dyspnea	Yes	Yes
Leukopenia	Possibly	No
PWP (pulmonary wedge pressure)	Normal	Increased
BNP (brain natriuretic peptide)	<200 pg/mL	Greatly elevated
Blood pressure	Usually hypotension	Usually hypertension
Increased vascular congestion/heart size	No	Yes
Diuresis	Hypoperfusion	Resolution of symptoms

Red Cell Storage

RBC anticoagulant-preservative solutions are approved for use by the FDA based upon testing to demonstrate a minimum RBC recovery of 75%, 24 hours after transfusion and less than 1% hemolysis in the RBC unit. However, there is no requirement to assess the clinical efficacy of an RBC transfusion. As a result, hospitals typically transfuse the "oldest" units in inventory first as not to outdate an expensive, often limited resource. Throughout storage, red cells acquire

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deleterious metabolic and structural changes which include increased levels of lactate, pH less than 6.5, reduced adenosine triphosphate and nicotinamide adenine dinucleotide levels, depletion of 2,3-diphosphoglycerate, impairment of sodium and potassium exchange, increased oxidative stress by oxidation of Hb to methemoglobin, disruption of the RBC cytoskeletal membrane, and microvesicle formation [72]. However, the association of this red cell storage lesion with clinical outcomes remains controversial. Initial observational studies have demonstrated associations of the age of RBC with risk for infection, thromboembolic events, multiorgan failure, increased ventilator time, increased ICU and hospital length of stay, and increased mortality [73]. Unfortunately, most of these studies were fraught with bias and confounding. As a result, several large, randomized, controlled clinical trials have been completed [74–78]. The results of the completed studies are summarized in Table 20.7, but none have found a difference in clinical outcomes following transfusion of fresh or old RBC across a variety of clinical settings.

Table 20.7 Key randomized, controlled clinical trials investigating clinical outcomes with red cell storage

Study	Number of subjects randomized	Study population	RBC randomization	Mean age of blood (fresh vs standard)	Percent nonconformance to RBC group	Primary outcome
Fergusson et al. [74]	377	Premature infants with a birth weight less than 1250 grams and required one or more RBC transfusions	7 days or less vs standard practice (dedicated donor unit per patient up to the expiration of the unit)	5.1 days vs 14.6 days	15.2% in fresh group, 0% in standard	52.7% of infants in the fresh RBC group compared to 52.9% in the standard RBC group experienced composite primary outcome (mortality and major neonatal morbidities associated with acute organ dysfunction or failure)
Steiner et al. [75]	1481	Individuals 12 years of age or older, weight 40 kg or more undergoing complex cardiac surgery with median sternotomy; patients 18 and older were required to have a likelihood of receiving a RBC transfusion of 60% or more during or within 24 hours of surgery	10 days or less vs 21 days or more	7.8 days vs 28.3 days	11% in the fresh group and 13% in the standard group	No difference in MODS or mortality

(contnued)

Table 20.7 (continued)

Study	Number of subjects randomized	Study population	RBC randomization	Mean age of blood (fresh vs standard)	Percent nonconformance to RBC group	Primary outcome
Lacroix et al. [76]	2510	Critically ill adults who had a RBC transfusion ordered within 7 days of ICU admission and were expected to require mechanical ventilation for at least 48 hours	8 days vs standard issue	6.1 days vs 22 days ($p < 0.001$)	16% in the fresh group, 0% in standard	No difference in 90-day mortality between fresh (37%) and standard (35.3%) RBC transfusion
Heddle et al. [77]	31,497	Adult hospitalized patients who required a RBC transfusion	Freshest RBC vs oldest RBC available	13.0 days vs 23.6 days ($p < 0.001$)	protocol required a minimum 10-day difference in mean red cell storage duration between treatment groups	No difference in in-hospital mortality between short-term (9.1%) and long-term (8.7%) stored RBC
Cooper et al. [78]	4994	Critically ill adults who had an anticipated ICU stay of at least 24 hours, and in whom the medical staff had decided to transfuse one or more RBC	Freshest, compatible, allogeneic RBC vs the oldest, compatible RBC	11.8 days vs 22.4 days ($p < 0.001$)	1.5% in the short-term, 0.5% in the long-term group	No differences in 90-day mortality between short-term (24.8%) and long-term (24.1%) stored RBC

RBC red blood cell, *MODS* multiple organ dysfunction syndrome

Summary

Transfusion is one of the most commonly performed procedures in hospitalized patients and a basic understanding of RBC transfusion is important for all ordering clinicians. This chapter reviewed blood product collection, manufacturing, storage, pretransfusion testing, and selection of appropriate RBC for transfusion including RBC modifications. Transfusion-related adverse events are not infrequent and clinicians should be aware of the noninfectious hazards of transfusion, especially TRALI and TACO. Last, this chapter which addressed two ongoing controversies regarding RBC transfusion thresholds and clinical outcomes associated with red cell storage were reviewed. Transfusing in the setting of hemorrhagic blood loss adds additional challenges including the utility of whole blood transfusion and optimum transfusion ratios, which are discussed in Chaps. 25 and 28, respectively.

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Plasma Transfusion

21

Jason B. Brill, Bryan A. Cotton, and Ryan A. Lawless

Introduction

Early administration of plasma to the massively hemorrhaging patient combats trauma-induced coagulopathy, decreases total blood product usage, and improves survival rates. Uncontrolled hemorrhage still accounts for approximately 40% of trauma-related deaths, in addition to 20–40% of deaths following hospital admission [1, 2]. As hemorrhage continues, patients develop hypovolemic shock in addition to multiple systemic changes resulting in trauma-induced coagulopathy (TIC). TIC is strongly associated with mortality in the trauma population [3–6], but prevention is possible with early plasma-directed therapy. This approach also decreases total transfusion requirements, as hemostasis and thus homeostasis are more readily achieved. Similarly, endotheliopathy of injury can be reversed or prevented with early plasma administration [7]. Plasma also appears to have neuroprotective effects [8]. In this chapter, we discuss the role of plasma in the resuscitation of the traumatically injured patient.

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Demographics and Usage

The most recent nationwide data regarding blood products in the United States are derived from the 2017 National Blood Collection and Utilization Survey (NBCUS). The survey has been conducted biennially since 1997 by the Centers for Disease Control and the Department of Health and Human Services. In the 2017 report, a total of 3,210,000 units of plasma were distributed and 2,318,000 transfused. This represents a 13.8% decrease from the previous NBCUS, a trend that has continued since 2011 [9, 10]. Improved utilization of the blood supply and more exacting guidelines regarding its use have been proposed as causes of this shift, a reversal of steady increases since the origination of the survey. The overwhelming majority of units were collected and produced by blood centers with less than 7% derived from hospitals. The median cost of a unit of plasma was $50–51, figures that are also declining [11].

Types of Plasma

Plasma is the aqueous portion of blood that contains coagulation factors, fibrinolytic proteins, albumin, immunoglobulins, and up to 6000 individual proteins. It is derived from whole blood or apheresis collection. Once collected, plasma can be frozen for storage or kept nonfrozen for

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immediate use. If frozen within 8 hours of phlebotomy, the product is labeled as fresh frozen plasma (FFP). If frozen after 8 hours from the time of phlebotomy but within 24 hours, it is labeled as plasma frozen within 24 hours (FP24). When FFP and FP24 are mobilized from the blood bank, they are thawed in a water bath to create thawed plasma (TP), which can be stored in liquid form for up to 5 days prior to transfusion. Liquid plasma (LQP) is derived from whole blood and is never frozen. The concentration of labile coagulation factors, namely, factors V, VII, and VIII, is affected by these various storage techniques, which will be further described in the next section.

Less commonly available products include "plasma, cryoprecipitate reduced" and "plasma frozen within 24 hours after phlebotomy held at room temperature up to 24 hours after phlebotomy." Plasma, cryoprecipitate reduced is the supernatant plasma remaining after the removal of cryoprecipitate from thawed FFP, but this is generally used for transfusion or plasma exchange in cases of thrombotic thrombocytopenic purpura. These forms are mentioned here only for completeness. Dried plasma, typically lyophilized or freeze-dried from FFP and later reconstituted for transfusion, is not currently approved for use in the United States, although several products are available elsewhere in the world. The characteristics of the different plasma preparations typically available for transfusion are summarized in Table 21.1.

A 2013 survey on transfusion practices at Level I and II trauma centers using data from the American College of Surgeons Trauma Quality Improvement Program reported that the types of plasma used at 90 centers were as follows: 78% thawed FFP or FP24, 16% thawed FFP/FP24 or LQP, and 7% LQP [12].

Fresh Frozen Plasma

FFP is prepared either by separating the red blood cells and platelets from whole blood with centrifugation or by apheresis. The plasma is then frozen at −18 °C or colder and stored until needed for transfusion. The freezing process must occur within 8 hours of donor phlebotomy. FFP contains high levels of all coagulation factors including the labile factors V and VIII. Whole blood yields approximately 200–250 mL of FFP, whereas apheresis collection yields 400–600 mL. The components are diluted approximately 8–20% by the anticoagulant, a mixture of citrate, phosphate buffer, and dextrose [13, 14]. Normal levels of factors V and VIII are found in FFP [15]. However, different blood groups yield different concentrations of coagulation factors, confounding efforts to standardize therapy. This variance is greatest in blood group O, which provides 30% less factor VIII and von Willebrand factor than other blood groups [14].

Table 21.1 Characteristics of differing forms of plasma for transfusion

	Derivation	Yield	Storage	Preparation time
Fresh frozen plasma	Centrifugation of whole blood or apheresis	Centrifugation—200–250 mL Apheresis—400–600 mL	Frozen at −18 °C within 8 hours of phlebotomy	20–40 min water bath thaw
Plasma frozen within 24 hours	Centrifugation of whole blood or apheresis	Centrifugation—200–250 mL Apheresis—400–600 mL	Frozen at −18 °C between 8 and 24 hours of phlebotomy	20–40 min water bath thaw
Thawed plasma	FFP or FP24 thawed and not transfused within 24 hours	Centrifugation—200–250 mL Apheresis—400–600 mL	1–6 °C for 4 days following thaw	Immediately available
Liquid plasma	Centrifugation of whole blood	n/a	1–6 °C for up to 30 days	Immediately available
Dried plasma	Lyophilization or freeze-drying	200 mL	Sterile container	2–10 min reconstitution

FFP must be thawed in a water bath between 30 and 37 °C prior to transfusion, a process requiring 20–40 minutes. Furthermore, breakage of bags occurs in the water bath in approximately 10% of cases, further delaying delivery to the bedside [16]. This can lead to a hazardous delay in transfusing a patient in extremis. Once thawed, FFP must be transfused within 24 hours or else relabeled as TP.

American Association of Blood Banks (AABB) indications for FFP transfusion include: [15]

1. Preoperative or bleeding patients with multiple coagulation factor deficiencies
2. Massive transfusion with clinically significant coagulopathy
3. Reversal of warfarin effects in patients who are bleeding or undergoing a procedure without enough time for vitamin K reversal or in patients needing transient reversal
4. Thrombotic thrombocytopenic purpura transfusion or plasma exchange
5. Selected coagulation factor deficiencies for which no specific concentrate is available
6. Rare specific plasma protein deficiencies when recombinant products are not available

The AABB also recommends against the utilization of FFP when a coagulopathy can be corrected by a more specific therapy, such as vitamin K, prothrombin complex, or specific coagulation factors. FFP is not the optimal therapy when complete reversal of warfarin is desired and should not be used when other volume expanders would suffice.

Plasma Frozen Within 24 Hours

Plasma collected via phlebotomy or apheresis and frozen between 8 and 24 hours following collection becomes FP24. When derived from whole blood, a unit of FP24 yields a volume of 200–250 mL. An apheresis unit of FP24 yields 400–600 mL. FP24 contains high levels of stable coagulation factors and slightly diminished levels of the labile factors V, VIII, and protein C due to the delay in freezing from donor collection time [15]. FP24 undergoes the same thawing process as FFP and also must be transfused within 24 hours of returning to the liquid state. As with FFP, if not transfused by that time, FP24 can then be relabeled as TP. The indications and contraindications for FP24 utilization are identical to FFP (see the section above). FP24 should not be used when the sole replacement of factors V and/or VIII is necessary [16].

Thawed Plasma

TP is the liquid form of FFP or FP24 following a thaw at 30–37 °C if the unit is not used within 24 hours of thaw time. It is then stored at 1–6 °C to be used for up to 4 days following the initial 24 hours post-thaw period. The levels of stable factors remain close to those of FFP and FP24 even at the 5-day point after the time of thaw. However, there is a decline in the levels of labile factors, most significantly factor VIII [13]. Viscoelastic testing also indicates slower thrombin generation after 5 days of storage [17]. AABB indications for transfusion of TP differ slightly from those of FFP and FP24, including [15]:

1. Preoperative or bleeding patients with multiple coagulation factor deficiencies
2. Initial treatment in patients undergoing massive transfusion with clinically significant coagulopathy
3. Reversal of warfarin effects in patients who are bleeding or undergoing a procedure without enough time for vitamin K reversal or in whom only transient reversal is needed
4. Thrombotic thrombocytopenic purpura transfusion or plasma exchange.

Because labile factor concentration is variable, TP is not recommended for the management of isolated or specific coagulation factor deficiencies for which other products containing higher concentrations are available.

Liquid Plasma

LQP is produced from whole blood no later than 5 days after the 21-day expiration period of whole blood. It cannot be frozen. LQP is refrigerated at 1–6 °C for up to 30 days. The primary indication for LQP is massive transfusion, as the thawing time is inherently absent. Vitamin-K-dependent factors (factors II, VII, IX, and X) are relatively stable under approved storage conditions. Therefore, LQP is currently indicated in patients on warfarin therapy who are suffering massive hemorrhage. The labile factors (V and VIII) deplete over time during storage, making LQP less effective in patients with these specific deficiencies [14, 15].

The hemostatic profile of LQP, as determined by thrombelastography, calibrated thrombogram, and clotting factor activity, is better than FFP or TP. Furthermore, the levels of coagulation factors remain ≥88% of original levels out to 26 days, except for factors V and VIII [18]. LQP's superior capacity to generate thrombin and form clot may be explained by the presence of platelet microparticles in LQP not present frozen plasma, cold activation of coagulation proteins, the decline in protein S activity, or a combination thereof. It is known that the freeze-thaw process required for FFP and FP24 degrades proteins; however, it is not understood why certain factors are less tolerant than others.

Dried Plasma

Although no dried plasma product is currently approved by the Federal Drug Administration for use in the United States [19–21], the earliest report of treating shock with dried plasma dates back to 1938 [22]. By the time of the United States' entry into World War II, freeze-dried plasma was approved for use by the Council on Pharmacy and Chemistry of the American Medical Association. The kit designed for reconstitution contained the packages of dried plasma with bottles of sterile water [23]. Eventually, several million units were produced by the American Red Cross for distribution to Allied Forces. This method served as the standard for the resuscitation of war casualties through the war, until hepatitis transmission from the pooled transfusions was recognized [23, 24]. By the 1950s, the United States discontinued use, although the French Military Blood Institute (Centre de transfusion sanguine des armées (CTSA)) and German Red Cross (Deutsches Rotes Kreuz (DRK)) still produce dried plasma products. Numerous studies are underway to develop and approve a product for US military and civilian use, discussed at the end of this chapter.

In general, dried plasma is produced either by freeze-drying, also known as lyophilization, or by spray-drying. Lyophilization involves cryodesiccation, freezing the plasma under a vacuum to decrease the water content to 1–2%. In the French method (French lyophilized plasma, or FLyP), apheresis plasma from a maximum of 10 donors is pooled and leukoreduced. After photochemical viroinactivation, three liters of product is distributed into individual flasks, which are freeze-dried for 4 days [25, 26]. This process removes much of the bicarbonate in the solution. Lyophilized plasma is therefore alkalotic with a pH near 8, causing issues in animal studies but appearing well tolerated in humans [25, 27]. The German method of lyophilization licensed as LyoPlas N-w (German Red Cross Blood Service, West Hagen, Germany) sources plasma only from single donors, a change made in 2006 due to concerns over Creutzfeldt-Jakob disease transmission [28]. The other general method of production, spray-drying, atomizes LQP by using pressurized droplets exposed to heated gas in a drying chamber, followed by rapid cooling [29].

Although safety concerns led to the discontinuation of dried plasma in the United States after the Korean War, newer methods of viral detection and inactivation have vastly improved the safety profile of the donor pool. Transmission of the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is calculated at one in two million units [30]. Solvent/detergent treatments bind lipid-enveloped viruses, and photochemical processes bind and inactivate non-enveloped viruses with any other free nucleic acids. A treatment including a prion reduction

step, Octaplas LG (Octapharma, Lachen, Switzerland), now has FDA clearance [31]. These processes are examined in more depth in the section "Infectious Disease Transmission."

Dried plasma possesses similar coagulation parameters as LQP and thawed FFP. In an animal model, Shuja et al. found an insignificant decrease in the activity of factors II, VII, and IX [26]. The German Red Cross Blood Service has reported minimal degradation of coagulation factor activity for factor V, factor XI, fibrinogen, protein S, antithrombin, and plasminogen. Only factor VIII and von Willebrand factor lost more than 10% activity, with the latter particularly affected by prolonged storage at room temperature. Cold storage for 24 months also showed retained coagulation factor activity with the same factor VIII and von Willebrand factor reduction of 20–25% [32]. While standard solvent/detergent processes can degrade coagulation factors significantly, newer methods preserve factor activity [31]. Additionally, minimal effect was seen with the reconstitution of dried plasma with acidic buffers to counteract the alkalotic dried form [27].

Current Practices in Civilian Trauma Centers

Patients in hemorrhagic shock suffer from a multitude of physiologic derangements. The early administration of blood products has been shown beneficial throughout the literature. However, the reasoning behind plasma transfusion is not as simple as previously thought. Trauma patients lose whole blood; therefore, giving whole blood or its components back makes sense. However, reaching for packed red blood cells (RBCs) first does not necessarily accomplish the goal that the trauma community is trying to achieve: restoration of circulating volume. The transfusion of plasma does restore circulating blood volume while delivering coagulation factors to bleeding patients actively consuming those factors. Early administration of plasma, in the prehospital setting as well as in the emergency center, has been shown to improve acid-base status upon admission, decrease transfusion requirements following admission, and reduce mortality risk [33–35]. These protective benefits can be synergistic with other therapies. When used to correct traumatic coagulopathy, combinations of FFP with tranexamic acid or prothrombin complex concentrate are superior in improving acidosis and coagulopathy than when these agents are given without plasma [36].

Restoration of circulating coagulation factors is not the sole purpose for the transfusion of plasma in patients with hemorrhagic shock. With increasing understanding of systemic endothelial injury associated with massive trauma, treating the endotheliopathy of trauma has also become a goal for plasma transfusion [37]. This evolution of thought has been derived from insights into how hemorrhagic shock systemically affects physiology.

Endotheliopathy of Trauma

The endotheliopathy of trauma refers to the breakdown of the endothelial glycocalyx on the endoluminal surface of blood vessels, increasing permeability and decreasing their integrity. Multiple proteoglycans and glycoproteins comprise this endoluminal network, providing surfaces for interactions with glycosaminoglycans, neutrophils, and a host of other particles. The glycocalyx normally allows the plasma component of blood to interact with the vessel wall while maintaining a barrier to erythrocytes and leukocytes [38]. It has been hypothesized that injury to the endothelial glycocalyx leads to interstitial edema, inflammation, and tissue hypoxia [39, 40]. Kozar et al. demonstrated that the endothelial glycocalyx is systemically injured during hemorrhagic shock, manifested by shedding of syndecan-1, one of the endothelial glycocalyx's proteoglycans [41]. Patients arriving at trauma centers in hemorrhagic shock express elevated level of syndecan-1. Johansson et al. found an elevated admission syndecan-1 level in severely injured patients to be associated with inflammation, coagulopathy, and increased mortality [42].

These effects, fortunately, are reversible. The injured glycocalyx is partially repaired with the

transfusion of plasma, an effect not seen with crystalloid solution [38, 41, 43]. Animal data suggests a decrease in inflammation with transfusion of plasma when compared to infusion of albumin, artificial colloid, and crystalloid in a hemorrhage shock model [44–48]. Increases in alveolar thickness, capillary congestion, and cellularity were seen in a shock model compared to sham; the infusion of crystalloid worsened all three parameters, while the plasma transfusion group demonstrated improvement, suggesting attenuation of lung injury [49]. Shown in Fig. 21.1 are electron microscopy images of mesenteric venules showing the effects of shock and resuscitation on the endothelial glycocalyx. TP, even when stored at 4 °C for 5 days after thawing, remained superior to crystalloid infusion with respect to reparative capacity in an animal model [7]. LQP also blocks endothelial permeability as effectively as thawed FFP [50]. This indicates that a component of plasma, in soluble form, interacts with the endothelial membrane to restore the endothelial glycocalyx. Figure 21.2 shows the vascular injury caused by hemorrhagic shock through hypoxia leading to cell contraction and increased vascular permeability. Resuscitation with plasma decreases the inflammatory response, promotes endothelial repair, and leads to normalization of the endothelium. This leads to a decrease in vascular permeability, clinically seen as less edema.

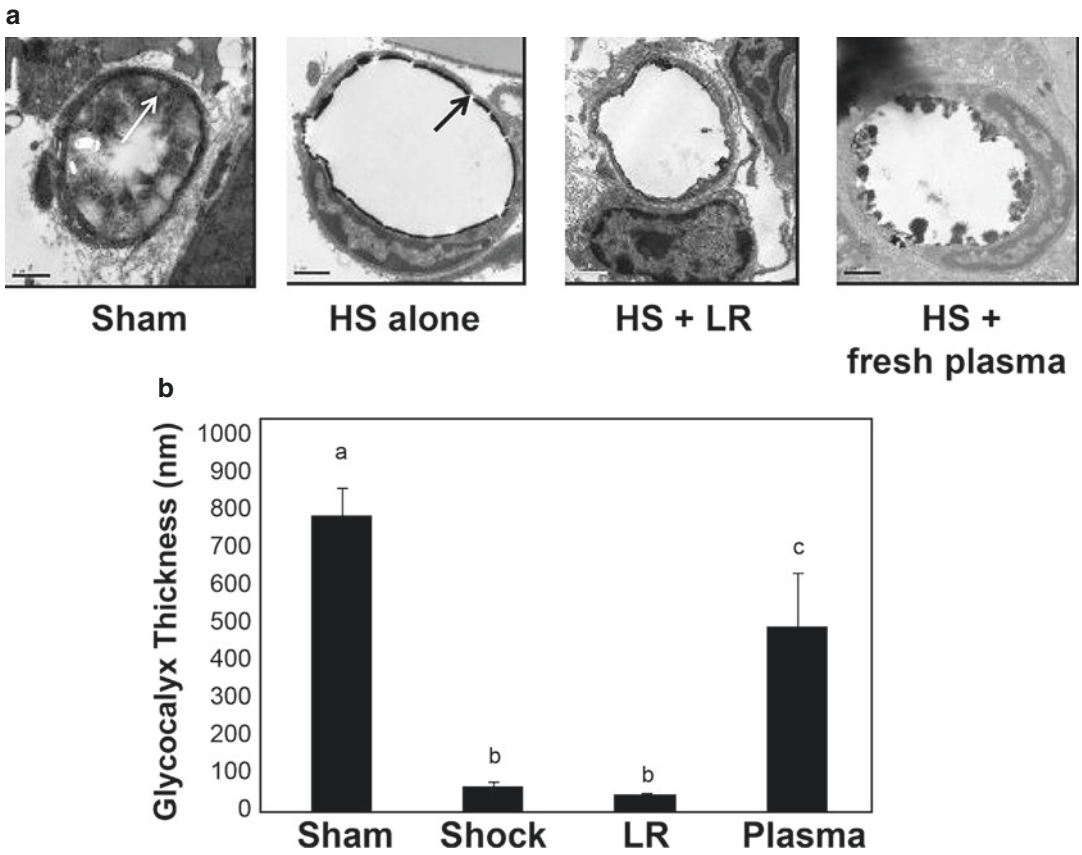


Fig. 21.1 Electron microscopy of mesenteric venules stained to reveal endothelial glycocalyx following hemorrhagic insult and resuscitation

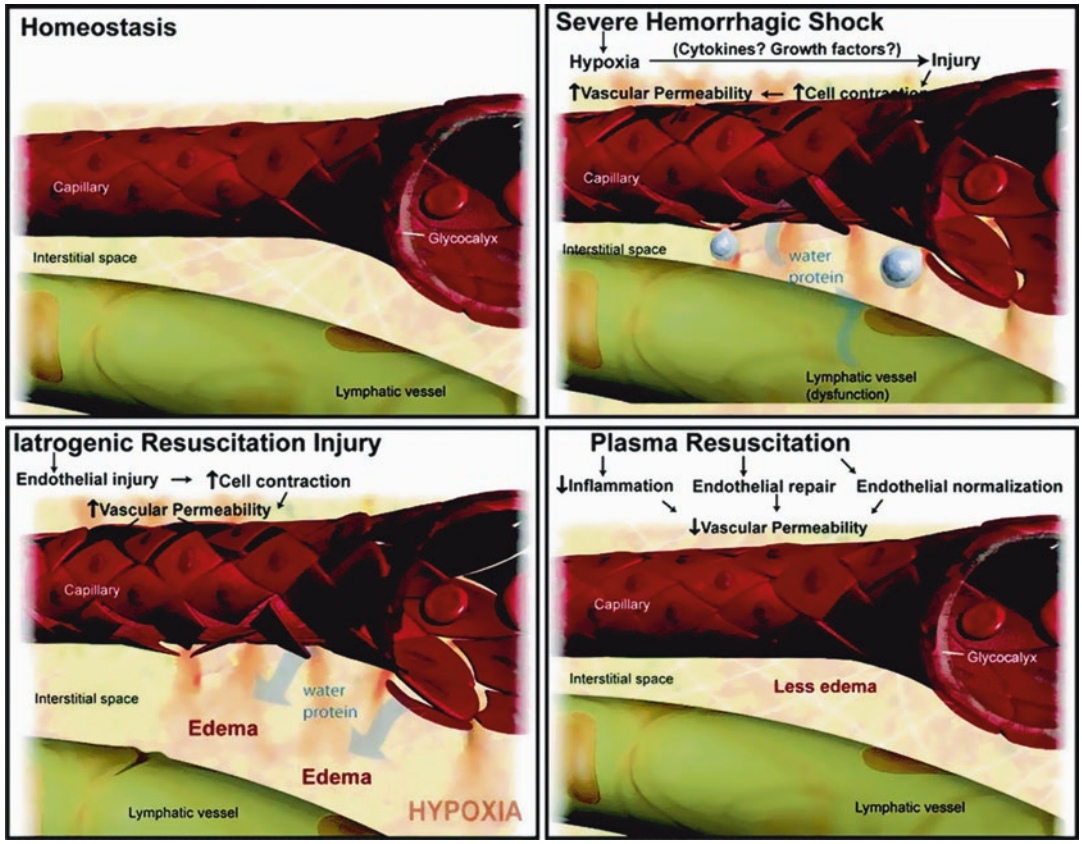


Fig. 21.2 Working biological model of the mechanism of action of fresh frozen plasma

Neuroprotective Benefits

Through the prevention and repair of endotheliopathy, in addition to other mechanisms still under study, plasma also confers neuroprotective effects in both animal models and human cohort studies. Transfusion of FFP in animal models of traumatic brain injury and hemorrhagic shock lessened the severity of injury based on the level of neurologic impairment and time to recovery. The purported mechanisms include improved cerebral perfusion, decreased excitotoxicity, and decreased mitochondrial dysfunction [51, 52]. Other porcine models demonstrated a reduction in the size of hemorrhagic lesions and intracranial swelling with plasma [8, 53]. Initial results from animal models using lyophilized plasma also show similar neuroprotection compared with FFP [54]. In humans, subgroup analysis of a large retrospective cohort found that early administration of plasma was associated with a survival benefit in patients with multifocal intracranial hemorrhage [55]. In another retrospective review, patients receiving prehospital TP had clinically significant improvement in neurologic outcomes versus patients receiving RBCs, based on Glasgow Outcome Scale-Extended and Disability Rating Score. The improvements extended to a median follow-up of 6 months [56].

The Underappreciated Benefits of Plasma

While attenuating or correcting trauma-induced coagulopathy through the restoration of clotting factors and endothelial protection are now considered primary mechanisms of action of plasma, it has other critical benefits. A single unit of plasma contains more than 400 mg of

fibrinogen, helping to address hypofibrinogenemia and fibrinogen dysfunction during the resuscitation of hemorrhage. In addition, plasma, likely through its high citrate content, serves as a tremendous acid-base buffer in hemorrhagic shock patients with severe acidosis. Traverso and colleagues demonstrated to be the best buffer available for resuscitation, with a buffering capacity 50 times that of standard crystalloid products [57]. Finally, these patients have also lost tremendous circulating blood volume, and plasma acts as a volume expander with high oncotic pressures.

Massive Transfusion Protocols

The institution of exsanguination protocols at major trauma centers has been shown to improve survival [58, 59]. Notably, early investigations did not specifically involve a balanced ratio resuscitation. Simply by initiating a protocol for the delivery of blood products to the emergency center for an exsanguinating patient, mortality improved. The activation of a "massive trauma protocol" delivered blood component therapy to the emergency center without the request of specific components by the trauma team. This was also demonstrated in the military environment with improved survival in both Iraq and Afghanistan [60, 61]. Furthermore, a decrease in the total amount of blood products transfused was seen [58, 59, 62, 63].

The majority of trauma centers store uncrossmatched RBCs in the emergency center. However, plasma often remains in the blood bank awaiting the activation of the massive transfusion protocol or a direct order from a physician. The lack of readily available plasma makes it difficult to achieve a high plasma-RBC ratio in an expedited fashion, which has been shown in multiple studies to decrease mortality [64, 65]. At one center, storing 4 units of thawed AB plasma next to the trauma bays, the time to first plasma transfusion was improved by 46 min. The study showed a decrease in the 24-hour transfusion of RBCs, plasma, and platelets and a significant decrease in mortality. Furthermore, a decrease in the frequency of activation of the massive transfusion protocol was seen following the implementation of TP in the emergency center [35]. Early administration of plasma in a massive transfusion protocol attains increased plasma-RBC ratios, which may reduce requirements for massive transfusion as currently defined [66].

Sourcing

While type AB plasma is traditionally considered the "universal donor," type A plasma has been used with increasing frequency as a safe alternative. Type A is more widely available, comprising 40% of the American population, as opposed to AB at 4%. This translates into 85% of trauma patients, those with A or O types, being able to receive A plasma with no additional risk [67]. In the remaining 15% of patients with type B and type AB, the risk of hemolytic reaction is limited by the generally low levels of secreted anti-B antibodies in most donors, especially in the United States [68]. To this end, some centers have started selecting for donors with low titers of anti-B antibody. This approach has been published with excellent safety records and no changes in mortality compared to AB plasma [67, 69–72].

Key Studies Involving Plasma

PROMMTT

The Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) study demonstrated that earlier transfusion of plasma, ideally within minutes of identification of hemorrhagic shock, achieved high plasma-RBC ratios and decreased 24-hour and 30-day mortality. This was evidenced by the three- to fourfold increased mortality risk associated with plasma-RBC ratios <1:2 [37, 73, 74]. Furthermore, gradual achievement of balanced transfusion ratios may not be as beneficial as early plasma transfusion. Initiating plasma transfusion early also led to a decrease in the total amount of RBCs trans-

fused during the initial 24 hours following admission [74]. PROMMTT clearly showed that earlier plasma was associated with improved survival; however, few patients received consistent ratios. There was significant concern that higher ratios would lead to increased hypoxia, acute respiratory distress syndrome (ARDS), and transfusion-related acute lung injury (TRALI). However, Robinson et al. showed that hypoxia was actually associated with excessive crystalloid infusion rather than plasma [75].

PROPPR

The PROMMTT study was followed by the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) study, which evaluated the effectiveness and safety of two transfusion strategies in patients with major trauma and severe bleeding. The PROPPR study was the first multicenter randomized trial comparing transfusion strategies with mortality as the primary endpoint. It provided evidence for the commonly held belief that balanced transfusion protocols were beneficial to severely injured patients in hemorrhagic shock. Although no significant differences in 24-hour and 30-day mortality were found between the two transfusion ratios investigated (plasma-platelet-RBC ratio of 1:1:1 versus 1:1:2), the early availability of blood products transfused within minutes of arrival in a balanced (1:1:1) ratio achieved hemostasis more often and decreased hemorrhage-related deaths within the first 24 hours following admission [76]. Furthermore, while the balanced transfusion group received significantly more plasma and platelets within the first 24 hours, no difference was found in the rate of 23 prespecified complications including ARDS, transfusion-associated circulatory overload (TACO), TRALI, and allergic reactions. Consistent with the biology of bleeding patients, the median time to hemorrhagic death in PROPPR was 2.3 hours. In a post hoc analysis, at 3 hours after admission, there was a significant mortality difference between the two groups. There was also lower hemorrhage-related mortality in the 1:1:1 group.

PAMPer

The Prehospital Air Medical Plasma (PAMPer) trial compared prehospital plasma resuscitation with standard care (i.e., no prehospital plasma transfusion) in 501 patients from 2014 to 2017 across nine trauma centers. Also designed as a multicenter randomized trial, two units of TP (either group AB or group A with a low anti-B antibody titer) were carried by participating air transport teams. As with PROPPR, a mortality separation developed at the three-hour mark [77]. The plasma group exhibited lower mortality, and this effect persisted to 30 days (23.2% vs 33%). Only five patients experienced transfusion-related adverse events labeled as possibly related to the trial treatment, all of which were minor allergic or other reactions. PAMPer serves as the first randomized trial to show the benefits of plasma in a prehospital setting with no increase in multiorgan failure, TRALI, nosocomial infection, or transfusion-related reactions.

COMBAT

Published concurrent with PAMPer, the Control of Major Bleeding After Trauma (COMBAT) trial also compared prehospital plasma resuscitation to standard care. Notable differences include a single-center design at the Denver Health Medical Center using all ground-based paramedics, as well as the transfusion of FP24 rather than TP. Sixty-five patients transfused two units of thawed FP24 were compared to 60 patients receiving saline, which was dosed according to the perceived need for resuscitation. No differences were seen in mortality at 28 days or in safety outcomes [78]. The authors suggested that in a nonurban setting with longer transport times than in Denver, plasma may have benefits that were not demonstrated in their setting. Concerns have been raised regarding the confounding effects of time needed to thaw frozen plasma en route, when median transport times were only 16–19 minutes [79]. In COMBAT, only 32% of the plasma group actually received the protocoled two units during transport, as opposed to

PAMPer, in which the plasma infusion was completed during air transport in 84.4% of the plasma patients with 89.1% receiving the planned two units. A combination of the COMBAT and PAMPer data later revealed that prehospital transport times do in fact have a significant effect on overall survival [80]. Pooling the data sets of the 626 patients between the two trials showed that while patients receiving standard care had increased mortality in transports longer than 20 minutes, the plasma group did not share this increased mortality. The post hoc analysis concluded that prehospital plasma was associated with reduced mortality when transport times are prolonged, with the primary benefit being observed in blunt trauma patients.

A Clinical Protocol of Plasma-Focused Resuscitation

The concept used at the Red Duke Trauma Institute at Memorial Hermann Hospital in Houston has been derived from the experiences gained on the battlefield, supported by military and civilian studies. The aggressiveness of plasma use in Houston was further driven by their experience with TRALI being increasingly rare and more likely associated with excessive crystalloid use [81]. In the prehospital setting, emphasis centers on the cessation of accessible bleeding. This occurs in parallel with hypotensive resuscitation utilizing whole blood or 1:1 ratio plasma-RBC units (with plasma being given first). Patients are identified as requiring a prehospital transfusion based upon the assessment of blood consumption (ABC) score [82]. Patients receiving blood in the prehospital setting have automatic activation of the institution's massive transfusion protocol (which often arrives at the trauma bay before the patient). In patients that have an ABC score <2 (1 point each for penetrating mechanism, systolic blood pressure <90 mmHg, heart rate >120 beats/min, and a positive focused abdominal assessment with sonography for trauma), rapid thrombelastography (rTEG) is used to guide blood product resuscitation on arrival to the trauma bay. Patients demonstrating shock, profound hypotension, and/or ABC scores ≥2 are started on the massive transfusion protocol and receive initially whole blood and then 1:1:1 ratio-driven resuscitation with early administration of LQP. The patient then proceeds to either the operating room or interventional radiology as indicated. Once surgical bleeding is controlled, the resuscitation converts to a guided, non-fixed-ratio approach utilizing rTEG and clinical response [83].

Adverse Effects and Events

Of all transfusable blood products, plasma, specifically FFP, is considered the most hazardous according to multiple studies into the mid-2000s. However, the overall risk remains low. The major risks include TRALI, TACO, infectious disease transmission, acute transfusion reactions, and leukocyte-associated reactions. It should be noted that RBCs, platelets, colloids, and crystalloids also carry well-described deleterious effects.

Transfusion-Related Acute Lung Injury

TRALI manifests as hypoxia, pulmonary edema, pulmonary infiltrates with radiographic changes, fevers, and possibly hypotension within 6 hours of transfusion. The presentation is similar to ARDS; however, >80% of patients typically recover within a few days of symptom onset, and treatment is mainly supportive [84]. A consensus panel update in 2019 updated the 2004 definition and introduced the terms TRALI Type I (without an ARDS risk factor) and TRALI Type II (with an ARDS risk factor or with mild existing ARDS) [85]. TRALI remains a clinical diagnosis, though serologic testing is available.

TRALI is significantly associated with leukocyte alloantibodies found in donor plasma. These specific antibodies are found almost exclusively in postpartum female plasma and in donors who have previously received a transfusion. Some authors believe TRALI to develop in two steps. A predisposing condition must be present that incites the

release of cytokines leading to the attachment of neutrophils to the pulmonary capillary endothelium. The second step occurs with neutrophil priming, activation, and pulmonary injury [86]. In 2008, Eder et al. reported a reduction in the incidence of TRALI with the conversion to male-predominant plasma for transfusion [87].

While it is the most common cause of death from transfusion and the most frequent serious complication of FFP transfusion, the absolute risk of TRALI remains low [88]. The UK hemovigilance Serious Hazards of Transfusion (SHOT) report included a single case of TRALI in the entire country in 2018 [89]. The estimated risk is approximately 1 in 64,000 transfused units. The FDA reported that the 56 reported cases of TRALI in the United States represented 30% of all fatalities secondary to blood transfusions from fiscal years 2013 to 2017. Plasma was implicated in four cases and a plasma product may have been implicated in 16 other cases that received multiple transfusions. No plasma-associated cases were reported for 2016 or 2017. The number of cases of TRALI has significantly decreased following voluntary measures taken by the transfusion community [90]. These measures include rigorous testing and safety procedures performed by the blood-banking community, as well as conversion to male-predominant plasma donors. At the caregiver level, the dramatic decrease in crystalloid use during the initial 24 hours of resuscitation of hemorrhage has been associated with marked reductions in its occurrence [75, 81].

Transfusion-Associated Circulatory Overload

TACO occurs secondary to increased hydrostatic pressure resulting in pulmonary edema. This process can be indistinguishable from TRALI, and the two entities may in fact coexist [91]. The FDA reported that TACO represented 18% of transfusion-related mortalities for the fiscal year 2017. There has been an uptrend in mortality related to TACO over time. It is difficult to determine whether the uptrend is secondary to improved diagnostics or actual increase in incidence [90].

The incidence of TACO is not well described in the literature. Multiple retrospective reviews have reported an incidence of TACO from <1% to 11% [91, 92]. In Fig. 21.3, Gajic et al. designed an algorithm to determine the etiology of post-transfusion acute pulmonary edema [91]. The algorithm is based on the European-American ARDS Consensus Conference definitions of TRALI and integrates specific laboratory values into the decision-making process. Frequently, TACO is a post hoc diagnosis made once a patient responds to a specific therapy [92].

Infectious Disease Transmission

The transmission of infectious diseases has dramatically decreased with extensive donor screening and infectious disease testing. Nucleic acid testing sensitivity has improved leading to a decrease in transmission risk. This has led to an estimated risk of 1:1,467,000 for acquiring HIV, 1:1,149,000 for HCV, and 1:280,000 donations for hepatitis B virus [88, 93, 94].

Outside the United States, many blood centers utilize donor-retested plasma, pathogen-inactivated plasma, and pathogen-reduced plasma [95]. Donor-retested plasma are units that are quarantined until the donor submits a subsequent donation which tests negative for infectious disease. Pathogen-inactivated and pathogen-reduced plasma are prepared via the addition of chemicals (solvent/detergent, methylene blue, amotosalen, riboflavin, and UV light) to prevent the transmission of lipid-enveloped viruses [96, 97]. Two companies leading this technology are Terumo Medical Corp. (Somerset, NJ) and Cerus Europe B.V. (Amersfoort, Netherlands). The Cerus process was FDA approved in 2015.

The freezing process during preparation of plasma inactivates bacteria. Furthermore, bacterial contamination with the production of endotoxin prior to freezing is unlikely [88]. The most recent annual FDA report describes no bacterial infection transmitted through plasma transfusions [90]. The process of removing cellular components via filters also removes cell-associated bacteria, most protozoa, and cell-

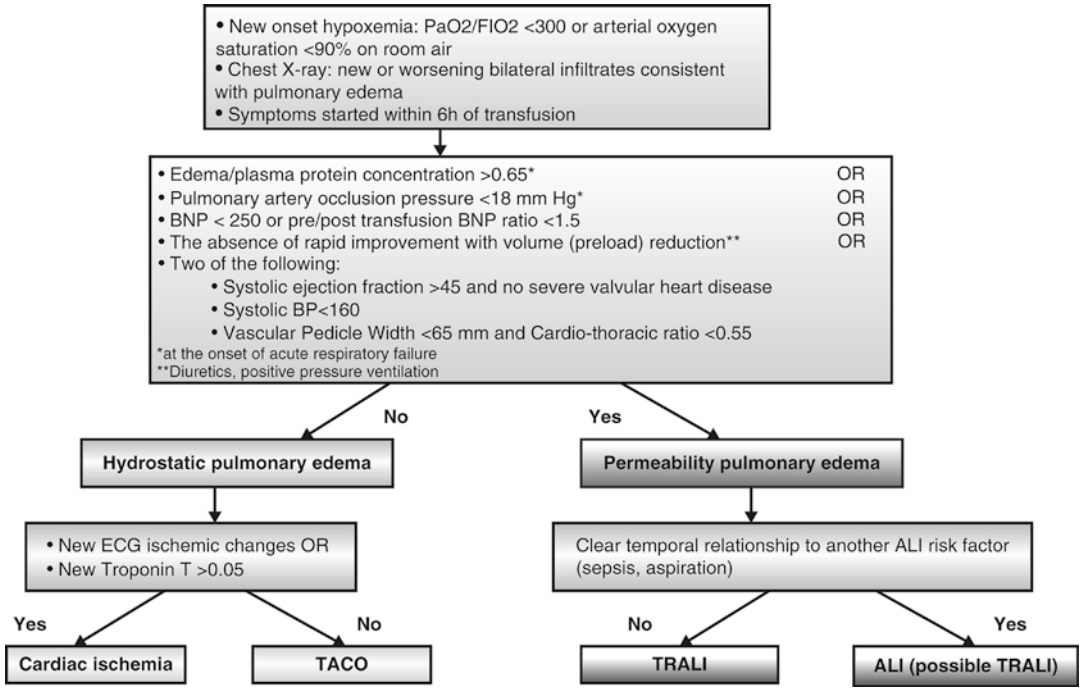


Fig. 21.3 Approach to posttransfusion pulmonary edema

associated viruses including malaria, cytomegalovirus, and human T- cell leukemia virus. Freezing does not remove the free viruses, including hepatitis A, B, and C, HIV 1 and 2, and parvovirus B19 [16].

Currently, no screening protocol exists for the detection of prion diseases, although techniques are being developed, such as protein misfolding cyclic amplification [98, 99]. Modern techniques of decreasing infectious transmission are ineffective against prion diseases. In the United Kingdom, three possible cases of variant Creutzfeldt-Jakob disease have been reported. Each case involved the transfusion of non-leukocyte-reduced RBCs [100]. There have been no reported cases of prion disease transmission following plasma transfusion; however, animal studies have shown it remains a possibility [101].

Febrile and Allergic Reactions

The 2018 SHOT Report documented 235 allergic, hypotensive, and severe febrile reactions. Only 11 cases were associated with plasma and/or cryoprecipitate, which were reported as a sum. These figures included five anaphylactic reactions. No deaths were related to any febrile or allergic reaction [89]. Moderate allergic reactions present with an urticarial rash, wheezing, and other symptoms not severe enough to be termed anaphylactic, and they occur in 1–3% of transfusions. Anaphylaxis includes bronchospasm, angioedema, severe hypotension, and cardiovascular collapse, a very rare occurrence [15, 89, 90, 92, 96].

Other than human immunoglobulin A (IgA) and haptoglobin, the proteins involved in acute transfusion reactions are unknown and generally unpredictable. For patients known to have an IgA sensitivity, plasma obtained from patients with IgA deficiency is available for transfusion [102].

Leukocyte-Associated Reactions

Leukocyte-associated reactions following plasma transfusion are also rare. The freeze-thaw process yields a small percentage of viable leukocytes prior to transfusion [103, 104]. The presence of

leukocytes leads to febrile nonhemolytic transfusion reactions, transfusion-associated graft-versus-host disease, and transmission of leukocyte viruses [96]. Following the freeze-thaw process, nonviable leukocytes release mediators that may contribute to febrile nonhemolytic transfusion reactions. These reactions are generally clinically insignificant and resolve quickly with supportive therapy [89, 96]. Graft-versus-host disease requires viable leukocytes to be transfused and then engraft and proliferate in the host patient. This is a rare transfusion reaction among all blood components transfused and has yet to be reported with FFP transfusion. Therefore, irradiation of FFP is not currently recommended [15].

Future Endeavors in Plasma Resuscitation and Research

The utilization of plasma as the primary resuscitation fluid has been the subject of multiple retrospective studies, a large prospective observational study, a recent randomized trial, and numerous other cohort studies. The benefits of balanced transfusion with respect to hemorrhagic mortality in the trauma population have been reported. Current randomized studies are evaluating the utility of plasma in the prehospital arena. Future research may yield an approved dried plasma product that eliminates the logistic constraints of frozen and liquid plasma.

Rationale

In order to achieve a more balanced transfusion goal set forth by multiple military and civilian studies, an immediate and plentiful supply of plasma is necessary. This becomes difficult in centers where TP is unavailable and in austere environments where cold chain storage and transportation is a limiting factor. As a result, much research and development have gone into the production and distribution of alternative plasma products suitable for human transfusion. Achieving the goal of 1:1:1 balanced resuscitation is often difficult in environments where a large and rapidly accessible supply of plasma is not available [105]. As described previously, FFP and FP24 require frozen storage and rewarming under very controlled conditions. TP and LQP require storage under refrigerated conditions with relatively short shelf lives. This makes the rapid (within minutes of requirement) administration of plasma difficult at most civilian institutions and sometimes impossible for military personnel.

Recent and Ongoing Studies Using Dried Plasma

These factors have led to the recent redevelopment of dried human plasma. As previously mentioned, lyophilized plasma is not a new concept, as it was first developed and introduced into practice during World War II. The French military has utilized lyophilized plasma with a well-documented hemovigilance program since 1994. No adverse effects of infectious transmissions have been reported with the transfusion of more than 1100 units [25]. In 2011, Martinaud et al. described the utilization of French lyophilized plasma intensive care units in Afghanistan while caring for casualties from coalition forces [106]. The product was described as easy to use, reconstituting within 10 minutes to provide 210 mL of fluid with hemostatic and volume expansive properties. A total of 236 units were delivered without a single adverse event reported. Israeli Defense Forces also utilize German LyoPlas N-w as a protocolized resuscitation fluid in the military prehospital setting. Using these dried products facilitates balanced transfusion from the point of initial resuscitation without playing "catch-up" after FFP thaws [107–109]. British helicopter transport has also shown decreased time to transfusion and the need for RBC transfusion in units carrying LyoPlas N-w [110].

French lyophilized plasma is approved by the Department of Defense, FDA, and White House for selected units of US Special Forces. In addition to the French, German, and Israeli militaries, dried plasma products are utilized in South Africa

and by the Norwegian military and civilian aeromedical services [107]. Further investigation for utilization in US civilian emergencies and ABO group universality are necessary, which may allow plasma resuscitation to occur in environments not conducive to current plasma storage [19, 20, 33].

Multiple products are under development in the United States with this goal in mind. HemCon Medical Technologies, Inc. (Portland, OR) started development in 2008 of lyophilized plasma sourced from single donor FFP under contract from the US Army Medical Materiel Development Activity (USAMMDA) and US Army Special Operations Command [111]. At the same time, the Office of Naval Research funded Entegrion, Inc. (Research Triangle Park, NC) with the goal of producing a pooled AB spray-dried plasma product named Resusix. This product uses a solvent/detergent process from Octapharma (Lachen, Switzerland) that not only controls lipid-enveloped viruses but also removes immunogenic lipids, cellular debris, and proinflammatory microparticles. Resusix is currently in Phase II clinical trial development [112, 113]. In 2012, the Biomedical Advanced Research and Development Authority funded the development of spray-drying technology with Velico Medical Technologies (Beverly, MA). The product called FrontlineODP (OnDemandPlasma) employs a decentralized manufacturing model, which would allow greater flexibility in individual blood centers quickly preparing dried plasma [56]. In 2014, Vascular Solutions, a subsidiary of Teleflex Corp (Limerick, PA), was awarded the USAMMDA Cooperative Research and Development Agreement previously with HemCon. Their product, RePlas, started Phase I clinical trials in 2017 [31, 114]. Terumo BCT, Inc. (Lakewood, CO) is developing another decentralized lyophilized plasma production process [115].

Further efforts may yield a plasma product with all the advantages of early plasma transfusion in trauma without the logistic difficulties of frozen storage and transport, thawing delay, and short shelf lives after thawing. Dried plasma development, while currently focused on military and austere environments, may progress to replace FFP in a multitude of settings such as prehospital transport, smaller hospitals, and mass casualty responses.

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Cryoprecipitate/Fibrinogen Concentrate Transfusions

22

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Rationale for Fibrinogen Supplementation in Trauma Hemorrhage

Fibrinogen is the key procoagulant factor needed for stable clot formation and effective hemostasis but falls rapidly and significantly during trauma hemorrhage [1] in association with increased fibrinolysis [2]. Fibrinogen metabolism is altered in trauma-induced coagulopathy with hypothermia and acidosis producing differential effects on fibrinogen synthesis and breakdown rate [3]. As the primary substrate of blood clots, some will be consumed during clot formation with some lost via direct fibrinogenolysis. Low fibrinogen levels at hospital admission are independently associated with in-hospital, 24-hour and 28-day mortality [4–6]. Patients have a greater than threefold increase in the odds of dying with a fibrinogen level less than <1 g/L compared to those with a normal fibrinogen concentration (1.5–3.5 g/L) [6]. Despite massive hemorrhage protocols (MHPs), there are often significant delays in the administration of the principal source of fibrinogen replacement which in the United Kingdom, North America, Australia, and many other countries is the frozen blood component – cryoprecipitate. Death from hemorrhage typically occurs early with a median time to death of 1.65 h [7], and therefore, many patients may die before receiving a concentrated fibrinogen product unless the trauma system has a license for use of an alternative fibrinogen replacement product, i.e., fibrinogen concentrate.

Although low fibrinogen levels are associated with poor outcomes, the threshold considered as critically low is not well-established. Traditionally, a cut-off of <1.0 g/L was used as the critical threshold. A nonlinear relationship has been shown between the plasma fibrinogen level and mortality, and an inflection point for increased mortality occurs at 2.3 g/L [1]. This level is markedly higher than previously acknowledged and suggests fibrinogen should be replaced earlier and more readily during bleeding. Precisely what threshold should be used to trigger fibrinogen replacement is yet to be conclusively defined and as such guidelines vary internationally (see Guidelines section "Guidelines & Thresholds for Triggering Fibrinogen Replacement"). Clinical data from

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uncontrolled observational studies suggest that fibrinogen supplementation may improve outcomes for trauma hemorrhage, by improving clot strength and reducing blood loss, which is associated with increased survival in both military and civilian settings [8]. To date, clinical trials of fibrinogen supplementation in trauma have been limited to relatively small feasibility or pilot randomized control trials (RCT), with none powered for a primary mortality end point, although the large, international CRYOSTAT-2 RCT (ISRCTN14998314 – see below) that is in progress is designed to answer the question of whether early high-dose fibrinogen supplementation improves survival from trauma hemorrhage (www.cryostat2.co.uk).

Product Details: Cryoprecipitate and Fibrinogen Concentrate

Cryoprecipitate is manufactured by slowly thawing fresh frozen plasma at 1–6 °C which precipitates out cryoproteins: FVIII, fibrinogen, FXIII, fibronectin, and von Willebrand factor. The cryoproteins are then centrifuged and resuspended in a reduced volume of plasma (20–60 mL) to form a unit that is stored at freezing temperatures (see Fig. 22.1). The shelf life while frozen varies between countries but is usually ~24 months and when thawed is 4 h. The starting plasma can be from either a whole blood donation or an apheresis collection. There is a considerable range per unit, approximately 200–1500 mg fibrinogen per bag due to donor variability, which is the primary reason for pooling units to reduce variation in fibrinogen content of cryoprecipitate. Standardization of cryoprecipitate as a blood product varies widely between national blood services [9], but as an example, the UK cryoprecipitate specification states that 75% of units contain at least 140 mg of fibrinogen and 70 iu of FVIII [10]. The cryoprecipitate is either distributed to hospitals pooled (usually 5–6 units) for immediate use after thawing or individual units are pooled prior to use in hospital. The preparation time for the product is 20–30 min for thawing and labeling. The final volume of an adult dose of two pools (10–12 units) is approximately 400 mL [10] and on average 4 g of administered fibrinogen raises the fibrinogen plasma level by 1.0 g/L [11].

Cryoprecipitate contains in addition to fibrinogen a number of other key coagulation factors – factor VIII, von Willebrand factor, factor XIII, and fibronectin as well as platelet microparticles (see Table 22.1). It is unclear what role these additional coagulation factors play in overall

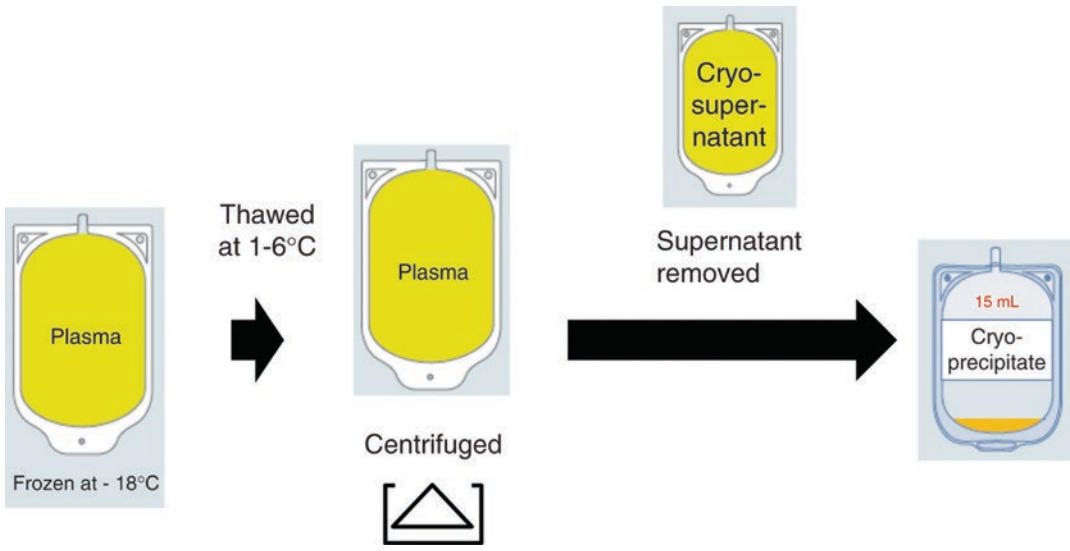


Fig. 22.1 Illustration of the production process for cryoprecipitate

Table 22.1 Content and roles of each of the components of cryoprecipitate

Substance	Specifics
Fibrinogen	This glycoprotein is synthesized by the hepatocytes and has a long half-life in the plasma of approximately 4 days. In contrast to factor VIII, fibrinogen activity remains stable with recovery of fibrinogen at 87% after 24 h of liquid storage, when compared to original content at the time of thawing
Factor VIII and von Willebrand factor (vWF)	Factor VIII and vWF represent approximately 5% of the total protein in cryoprecipitate. vWF performs two major functions in primary hemostasis: it mediates the adhesion of platelets to exposed subendothelium, and it stabilizes coagulation factor VIII in the plasma
Factor XIII	Factor XIII promotes clot stability by forming covalent bonds between fibrin monomers to enhance the mechanical strength of the fibrin clot and protect the clot from proteolytic degradation. Cryoprecipitate contains approximately 20%–30% of the original factor XIII of plasma
Fibronectin	Fibronectin is a dimeric α2-glycoprotein present at a concentration of 300 μg/mL in human plasma. It is thought to have opsonic activity assisting with the phagocytosis of particulate debris by the reticuloendothelial system. Cryoprecipitate is a rich potential source of fibronectin at 1500 μg/mL
Platelet microparticles	The process of preparing cryoprecipitate concentrates the platelet membrane microparticle content. The platelet membrane microparticle concentration of cryoprecipitate is 29-fold greater than the cryosupernatant plasma and 265-fold greater than the original plasma. Each clinical dose of cryoprecipitate (10 units) contains approximately 4×10^9 platelets in microparticle form. Microparticles can play active roles in thrombosis, inflammation, and vascular reactivity

hemostatic potential or indeed whether the presence of anticoagulant factors (antithrombin) or antifibrinolytic factors (alpha-2 antiplasmin) play an additional role, perhaps by "balancing" hemostatic and thrombotic processes. Fibrinogen is found in a number of blood components – plasma, platelet, and whole blood units – and therefore, patients will receive additional fibrinogen supplementation throughout hemostatic resuscitation if these are transfused. One unit of 250 mL of plasma contains approximately 400–900 mg of fibrinogen or 2.5–3.0 g per 1000 mL.

Pathogen-Inactivated Cryoprecipitate

There are three pathogen inactivation (PI) systems for plasma: methylene blue (Theraflex), amotosalen (Intercept), and riboflavin (Mirasol). All these systems are based on the addition of a photosensitizer to plasma followed by exposure to ultraviolet light and then the removal of the photosensitizer (except for Mirasol). All PI systems reduce the level of coagulation factors in cryoprecipitate – in general, the losses for FVIII and fibrinogen are approximately 30%. As a result of the loss in fibrinogen, the pooling process in some countries is increased from five to six donor units in order to meet the specifications [10].

Fibrinogen Concentrate

Cryoprecipitate has been discontinued in some countries and replaced with virally inactivated fibrinogen concentrates due to concerns regarding viral transmission and other blood transfusion-related side effects such as allergic reaction, fluid overload, and TRALI (transfusion-related acute lung injury) among others. Fibrinogen concentrate is a heat-treated, lyophilized fibrinogen powder made from pooled human plasma. It is a standardized product and has been available for use for over 50 years. RiaSTAP™ is the most commonly available product has been used for many years in several European countries under the name Haemocomplettan® P. Each vial contains between 900 and 1300 mg fibrinogen, 400 and 700 mg human albumin, and other constituents which aid the dissolution of the powdered fibrinogen into the solvent. It is reconstituted immediately before use with 50 ml of water for injections containing approximately 20 mg/ml

human fibrinogen. RiaSTAP® is indicated for the treatment of acute bleeding episodes in patients with congenital fibrinogen deficiency, including afibrinogenemia and hypofibrinogenemia, but it is the first-line agent for fibrinogen replacement therapy in trauma hemorrhage in many European countries. It is contraindicated in patients with known anaphylactic or severe systemic reactions to human plasma-derived products.

Proportion of Cryoprecipitate Issued to Hospitals in Trauma

Several audits on the appropriateness of cryoprecipitate use have been published. Canada audited the cryoprecipitate use at 25 hospitals including 603 orders for 4370 units of cryoprecipitate [12]. Overall, 12.3% were used for trauma resuscitation with a rise in fibrinogen from a median of 1.3–1.9 g/L. Similarly, an audit in 39 hospitals in the United Kingdom (423 episodes of cryoprecipitate infusion) found that 12% of adults and 6% of pediatric orders were for trauma care. The median dose in adults was 10 units, and the rise in fibrinogen observed in adults was 0.8 g/L and in children 0.9 g/L [13]. Lastly, an audit in Australia of 460 episodes of cryoprecipitate infusions at 23 hospitals found 9% of all cryoprecipitate used was for trauma resuscitation [14]. A report from 2011, again from Australia, noted a rising rate of cryoprecipitate use at a single trauma center study [15]. The rate increased from 0.03 units per patient in 1998 to 1.3 units per patient in 2008 for patients undergoing a massive transfusion (10 units in 24 h), of which 26% were trauma-related hemorrhages. The reason for the rise in cryoprecipitate use was not determined by the authors, although they speculated that thromboelastography (TEG)-guided hemostatic management may have been a contributing factor.

Clinical Use of Cryoprecipitate Transfusion in Trauma

The British Society of Hematology [16] recommends transfusion of two pools of cryoprecipitate if fibrinogen level is below 1.5 g/L, and current European guidelines [17] state a threshold of 1.5–2.0 g/L or viscoelastic signs of a functional fibrinogen deficit to trigger fibrinogen replacement therapy. The precise timing and ratio of fibrinogen replacement therapy to other blood components continue to vary widely between MHPs as highlighted in a 2018 national audit from the United Kingdom [18]. Two earlier large observational studies from the United States and the United Kingdom found similar and significant delays in the timing of cryoprecipitate during transfusion for major hemorrhage in trauma. In the UK study conducted at 22 hospitals, bleeding trauma patients waited for an average of 3 h for the first transfusion of cryoprecipitate [19], and in massive hemorrhage cases (RBC 10+ units), only half of the patients received any cryoprecipitate. The PRospective Observational Multicenter Major Trauma (PROMMTT) study group prospectively collected data on 1245 traumas patients in ten US level I trauma centers and were able to examine cryoprecipitate use [20]. To be enrolled in this study, the patients had to survive at least 30 min after injury, receive at least 1 red blood cell (RBC) unit, and have at least 3 units of hemostatic blood components within 24 h. Great variability in cryoprecipitate utilization was observed per center ranging from 7% to 82% of patients. The authors hypothesized that early and increased use of cryoprecipitate would save lives, but unfortunately, they could not detect any signal to support their primary study objective. Overall, 359 patients (29%) were administered cryoprecipitate in the first 24 h. The patients who were transfused cryoprecipitate were more severely injured and received more of every blood component type. Only the admission fibrinogen level was recorded which was below 1.5 g/L in 30% of cryoprecipitate transfused patients and 17% in non-transfused patients – the pre-transfusion fibrinogen levels were not recorded. Predictors of cryoprecipitate use were admission fibrinogen level under 1.0 g/L, lower hemoglobin, and pelvic bleeding.

In the PROMMTT study, the median number of cryoprecipitate units transfused was 10, and on average, this was administered 2.8 h (interquartile range 1.7–4.5 h) from arrival. On

average, eight RBC units (with a very wide interquartile range of 4–16 units) were transfused before the first dose of cryoprecipitate. Only 1% of patients were transfused cryoprecipitate before any plasma or platelets. In either study, it is unclear whether the long time to cryoprecipitate and the fact that no cryoprecipitate was given to many patients with hypofibrinogenemia was due to early death before infusion, an intentional clinician decision due to non-survivable injuries, or a long time delay in product preparation. In PROMMTT, cryoprecipitate use did not appear to increase, or decrease, survival; although perhaps used as a "last-ditch" as described at these trauma centers, it is not the optimal transfusion strategy. In summary, the findings from this and the UK studies demonstrate ongoing highly variable practice across major trauma centers of when to commence concentrated fibrinogen replacement therapy in bleeding patients and that prospective, randomized trials are required to inform decisions on the timing of cryoprecipitate administration during an MHP.

Clinical Use of Fibrinogen Concentrate (FC)

Retrospective data from small observational studies in Europe have reported improved outcomes and coagulation profiles with fibrinogen concentrate, used alone or in combination with prothrombin complex concentrates, and on the most part guided by viscoelastic coagulation tests, e.g., ROTEM or TEG. Fibrinogen concentrate lends itself for use in goal-directed therapy as it has a defined concentration, does not need to be stored in a blood bank, and can be administered quickly. To date, the only validated algorithm for coagulation management utilizing ROTEM or TEG in trauma hemorrhage [21] is that developed for the multicenter implementing Treatment Algorithms for the Correction of Trauma-Induced Coagulopathy (iTACTIC) clinical trial (NCT02593877). However, in centers that routinely use ROTEM or TEG and have access to fibrinogen concentrate, it is typically considered as first-line therapy in patients with impaired fibrin polymerization, i.e., a clot amplitude of <7 mm at 10 min after clotting time measurement in the FIBTEM assay (target FIBTEM CA10: 10–12 mm) [22] or equivalent parameters on the functional fibrinogen assay for TEG.

A matched pairs registry study from Germany of nearly 600 trauma patients found that the early use of fibrinogen concentrate was associated with a significantly lower 6-h mortality and an increased time to death but no reduction of overall hospital mortality [23]. In a 96-patient study from Ponschab et al. comparing pre- and post-administration of (4 g) fibrinogen concentrate, significant improvements in fibrin polymerization and overall clot function were measured by an increase in clotting time, alpha angle, and maximum clot firmness on ROTEM [22]. Clotting time is dependent not only on thrombin generation but also on the availability of substrate (fibrinogen) and, therefore, accelerated fibrin polymerization rate results in earlier clot formation. The same group from Austria has previously published data comparing FFP vs factor concentrate (including fibrinogen) therapy using a ROTEM-guided algorithm in trauma hemorrhage [24] compared to national registry data. In the 80 patients who received fibrinogen concentrate from their institution vs the 601 patients who received FFP in the national registry, they found similar mortality rates, but RBC transfusion was avoided in 29% of patients in the fibrinogen-PCC group vs 3% in the FFP group. Transfusion of platelet concentrate was avoided in 91% of patients in the fibrinogen-PCC group vs 56% in the FFP group. One possible explanation postulated by the authors for this avoidance in allogeneic blood products is the speed of correcting TIC with a product stored at room temperature in the ED vs a frozen product that requires thawing and portering from the blood bank. The average dose of fibrinogen concentrate administered was 6 g, and in an earlier study [24], the authors found that despite high doses of fibrinogen replacement, plasma levels were low normal at 24 h and only became supranormal (>4.5 g/L) at 72 h which is consistent with the acute inflammatory response after injury.

Impact of Fibrinogen Supplementation on Outcomes from Trauma Hemorrhage

Cryoprecipitate Transfusion

As yet, there are no large RCTs comparing fibrinogen replacement with cryoprecipitate vs placebo in trauma patients to determine the impact of more high-dose fibrinogen supplementation on clinical outcomes (CRYOSTAT-2 is scheduled to finish recruitment end of 2020 – see the section below). One small pilot RCT and five retrospective reports have attempted to determine the impact of more aggressive fibrinogen replacement on outcomes in pediatric and adult trauma, and these studies are described below. One limitation of all the retrospective studies is the issue of survivor bias in determining the true effect of higher volumes of cryoprecipitate when evaluating the outcome. Both plasma and cryoprecipitate require substantial time for preparation and, therefore, some patients may have died before having the opportunity to receive these frozen components. CRYOSTAT-1 was a pilot RCT in trauma hemorrhage [25] and demonstrated that it was feasible to deliver cryoprecipitate within 90 min of admission. The primary finding was that early cryoprecipitate therapy maintained blood fibrinogen levels above 1.8 g/L during resuscitation with a signal for reduced mortality (29% vs 10%) and has provided pilot data for the larger CRYOSTAT-2 trial (see the section below). Summary data from clinical trials in which cryoprecipitate was used for fibrinogen supplementation is available in Table 22.2.

The first retrospective report was published in 2008 by Stinger et al. [26]. The authors analyzed data from the US Joint Trauma Registry which included patients from two combat hospitals in Iraq between 2004 and 2005. Only patients who received ten or more RBC units or whole blood in the first 24 h were included (n = 252 of 5586 injured patients). They calculated the fibrinogen to RBC ratio, considering fibrinogen content from all blood components, including cryoprecipitate; unfortunately, they used 2.5 g per 10 units of cryoprecipitate, when probably the true content was double this estimate. Patients who received less than 0.2 g per RBC had an increased risk of death, but patients who received higher ratios of fibrinogen to RBC units did not improved survival (i.e., no dosage effect observed). The mortality rate was 52% in the lowest quartile compared to 24% in the highest quartile ($p < 0.001$).

The second report from Emory University [27] reviewed trauma patients who had received ten or more RBC units in the first 24 h. They assessed patients in two time periods (pre- and post-MHP implementation) with cryoprecipitate administered at the discretion of the clinical team in the pre-MHP era. In the post-MHP period, the hemorrhage protocol provided plasma in a 1:1 ratio with RBC units, with cryoprecipitate (20 units) provided in a pack of three units and then 10 units of cryoprecipitate with every other pack of six RBC units. Interestingly, cryoprecipitate use overall was the same in both cohorts, and they found no difference in mortality at 24 h and 30 days. They then pooled both time periods of patients and looked at the association between high cryoprecipitate ratio (≥1 unit per RBC unit), compared to a lower ratio, and mortality. A high ratio was associated with better 24 h (84% vs. 57%) and 30-day (66% vs. 41%) mortality, compared to a lower ratio.

The third report by Rourke et al. [4] reviewed the fibrinogen levels of all trauma patients between 2008 and 2010 (n = 517). The admission fibrinogen was greater than 1.5 in 78%, less than 1.5 g/L in 14%, less than 1.0 g/L in 5%, and less than 0.5 g/L in 3%. A low admission fibrinogen level was associated with a higher Injury Severity Score (ISS) and a greater degree of shock. Cryoprecipitate was administered at a median of 103 min (interquartile range 78–134 min) from arrival in the 39 (7.5%) patients who were administered cryoprecipitate. Fibrinogen levels were higher in survivors compared to non-survivors, but there was no difference in mortality for those given cryoprecipitate versus no cryoprecipitate at 24 h or 28 days. In multivariate analysis, fibrinogen at baseline was an independent predictor of mortality at both 24 h and 28 days; the odds of death was reduced by 0.22 during the first 28 days for every 1 g/L rise in base-

Table 22.2 Summary of trials

Study name (location)	Study title	Inclusion criteria	Comparator arm	Intervention arm	Primary outcome	Patients and results	Author/trial registration
CRYOSTAT-2 (UK & USA)	A multi-center, randomized, controlled trial evaluating the effects of early high-dose cryoprecipitate in adult patients with major trauma hemorrhage requiring major hemorrhage protocol (MHP) activation	Adult trauma patient (>16 years) *and* Deemed by the attending clinician to have on-going active hemorrhage *and* Requires activation of the local major hemorrhage protocol for management of severe blood loss *and* Has started or received at least one unit of any blood component	Major hemorrhage protocol only (RBC, FFP followed by platelets and cryoprecipitate)	Early cryoprecipitate (3 pools equivalent to 15 single units cryoprecipitate) within 90 min of admission in addition to the standard (local) major hemorrhage	All-cause mortality at 28 days	1568 participants Study in progress	ISRCTN14998314
FEISTY (Australia)	Fibrinogen Concentrate vs Cryoprecipitate in Traumatic Hemorrhage: A Pilot Randomized Controlled Trial	Adult trauma patient (>18 years) *and* Judged to have significant hemorrhage *or* Predicted to require significant transfusion with ABC Score ≥ 2 or by treating clinician judgment	Fibrinogen replacement using cryoprecipitate as per ROTEM-guided treatment algorithm [FIBTEM A5 $\leq$ 10 mm]	Fibrinogen replacement using fibrinogen concentrate as per ROTEM-guided treatment algorithm [FIBTEM $\leq$ A5 10 mm]	Time to administration of fibrinogen replacement from time of ROTEM analysis indicating fibrinogen supplementation is required Effects on fibrinogen levels during traumatic hemorrhage as measured by Clauss fibrinogen and FIBTEM	100 participants Study completed (results awaited)	NCT02745041 [34]

(continued)

Table 22.2 (continued)

Study name (location)	Study title	Inclusion criteria	Comparator arm	Intervention arm	Primary outcome	Patients and results	Author/trial registration
RETIC (Austria)	Reversal of Trauma Induced Coagulopathy Using Coagulation Factor Concentrates or Fresh Frozen Plasma	Adult trauma patients (18–80 years) *and* Major trauma (ISS > 15) *and* Clinical signs of ongoing bleeding or patients who are at risk for significant hemorrhage assessed and judged by the ED team in charge of patient *and* Presence of coagulopathy defined by ROTEM	Fresh frozen plasma (15 ml/kg)	Fibrinogen concentrate (50 mg/kg) if FIBTEM A10<7 mm Prothrombin complex concentrate (20 IE/kg) if EXTEM CT >90 sec and FIBTEM A10>7 mm FXIII concentrate (20 IU/kg) with second dose of fibrinogen concentrate	Multiple organ failure	100 participants Study terminated early for futility and safety reasons because of the high proportion of patients in the FFP group who required rescue therapy compared with those in the intervention group Multiple organ failure occurred in 29 (66%) patients in the FFP group and in 25 (50%) patients in the CFC group (OR 1.92 [95% CI 0.78–4.86], $p = 0{\cdot}15$)	Innerhofer et al [31]
FI in TIC (Austria, Germany, and Czech Republic)	A Multicenter Double-blind, Placebo Controlled, Randomized, Pilot Trial to Assess the Efficacy of Pre-hospital Administration of Fibrinogen Concentrate (FGTW) in Trauma Patients, Presumed to Bleed (FI in TIC)	Adult trauma patient ≥18 years *and* Major bleeding or occult bleeding with parameters of shock *and* Need for volume replacement therapy	Placebo (not specified)	Fibrinogen concentrate administered by helicopter emergency physicians prehospital and dosed according to body weight (1.5–6 g)	Change of the fibrinogen polymerization measured with FIBTEM Maximum Clot Firmness (ROTEM)	67 participants Study completed (results awaited)	NCT01475344 Narrative review [33]

FiiRST (Canada)	Fibrinogen in the Initial Resuscitation of Severe Trauma: A Randomized Feasibility Trial	Injured trauma patients who are at risk of significant bleeding defined as: Systolic blood pressure ≤ 100 mmHg at any time from the injury scene until 30 min after hospital admission *and* Red blood cell transfusion has been ordered by the trauma team leader	Saline 0.9%	6 g fibrinogen concentrate	Feasibility endpoint – proportion of patients receiving study intervention within first hour of hospital admission	50 participants 96% of patients received the intervention within 1 hour Plasma fibrinogen concentrations remained higher in the intervention arm	Nascimento et al [30]
CRYOSTAT-1 (UK)	A feasibility study for a multi-center, randomized controlled trial evaluating the effects of early administration of cryoprecipitate in major traumatic hemorrhage	Adult trauma patient (>16 years) *and* Deemed by the attending clinician to have on-going active hemorrhage *and* Requires activation of the local major hemorrhage protocol for management of severe blood loss	Major hemorrhage protocol only (RBC, FFP followed by platelets and cryoprecipitate)	Early cryoprecipitate (2 pools equivalent to 10 single units) in addition to standard massive hemorrhage protocol	Feasibility endpoint: Proportion of patients in the intervention arm who receive cryoprecipitate within 90 min of admission	43 participants 85% in intervention arm received cryoprecipitate within 90 min Median time 60 min (early cryopre-cipitate) vs 108 min (MHP alone) Fibrinogen concentrations were higher in the intervention arm above 1.8 g/L at all time-points during active hemorrhage	Curry et al. [25]
PRooF-iTH (Denmark)	Effect of Immediate, Pre-emptive Fibrinogen Concentrate in Patients With Trauma Hemorrhage Needing Hemostatic Resuscitation - A Randomized, Controlled, Double-blinded Investigator-initiated Pilot Trial	Adult trauma patient (≥18 years) *and* Initiated order of transfusion of at least one blood component within the 1st hour of arrival *and* Predicted to need transfusion package therapy during the initial resuscitation (first 2 hours)	Saline 0.9%	Single dose of fibrinogen concentrate when hemostatic resuscitation is deemed necessary by the clinician	TEG® Functional Fibrinogen maximum amplitude 15 min	40 participants Study completed (results awaited)	NCT02344069 [35]

(continued)

Table 22.2 (continued)

Study name (location)	Study title	Inclusion criteria	Comparator arm	Intervention arm	Primary outcome	Patients and results	Author/trial registration
No study name (Brazil)	Early Administration of Fibrinogen Concentrate in Polytraumatized Patients With Thromboelastometry Suggestive of Hypofibrinogenemia: A Randomized Feasibility Trial	Adult trauma patients (18–80 years) *and* Severe trauma (ISS > 15) *and* Hypotension (systolic blood pressure < 90 mmHg) *and* Tachycardia (heart rate > 100 bpm) Qualitative hypofibrinogenemia (FIBTEM A5 ≤ 9 mm)	Did not receive fibrinogen concentrate	Early fibrinogen concentrate (50 mg/kg)	Feasibility assessed by the proportion of patients receiving the allocated treatment up to 60 min after randomization	32 participants Study completed (results awaited)	NCT02864875

line fibrinogen. In an analysis of the fibrinogen-to-RBC-unit ratio, the odds of death during the first 28 days (conditional on survival to 12 h) was decreased by a factor of 0.91 for every 1 g increase in ratio over the first 12 h, with the fibrinogen dose calculated for all components transfused (not just the cryoprecipitate).

In the sole pediatric study, Hendrickson et al. from Emory University [28] reviewed 102 trauma patients between 2006 and 2010 who were transfused with at least one blood component in the first 24 h. Overall, 52% of patients had an abnormal fibrinogen level, 21% greater than 1.0 g/L, 20% less than 1.0 g/L, and 11% undetectable (48% had normal levels). In total, 25% of patients received cryoprecipitate. In multivariate analysis, the fibrinogen level was not statistically significant in predicting outcomes; only initial prothrombin time (PT), partial thromboplastin time (PTT), and platelet count remained significant in their model.

Lastly, the Military Application of Tranexamic acid in Trauma Emergency Resuscitation (MATTERs) II Study [29] reported data from the US and UK Joint Trauma registries for patients injured from 2006 to 2011 in Afghanistan. They included patients who had received at least 1 RBC unit in this retrospective analysis. The use of cryoprecipitate (and tranexamic acid (TXA)) was left to the judgment of the individual physicians. The dose administered was 10 units at a time. Overall, 1332 patients received at least 1 RBC unit, of whom 168 (12.6%) received cryoprecipitate. Due to its retrospective design, numerous imbalances were observed between their four groups (no TXA or cryoprecipitate, only TXA, only cryoprecipitate, or both); the most severely injured group (ISS) was the cryoprecipitate only group, and the cryoprecipitate/TXA group required four times the amount of RBC, plasma, and platelet units. The median dose administered was 2.1 and 2.3 units per patient for cryoprecipitate only vs. cryoprecipitate/TXA (1 unit in this report was a 5-unit pool). Surprisingly, mortality was lowest in the cryoprecipitate/TXA group (11.6%) compared to no cryoprecipitate/TXA (23.6%). The administration of cryoprecipitate was associated with OR for death of 0.61 (95% CI 0.42–0.89), with the cryoprecipitate/TXA combination being associated with a substantial reduction in the risk of death (OR 0.34, 95% CI 0.20–0.58).

Fibrinogen Concentrate

Several small trials both (see Table 22.2) in prehospital and in-hospital, all with <100 patients per study, have used fibrinogen concentrate as replacement therapy for fibrinogen, with coagulation results either conventional or ROTEM/TEG used as the primary end points. FiiRST [30], RETIC [31], and EFIT-1 [32] all demonstrated feasibility of early infusion of supplemental fibrinogen for the majority of patients with an associated rapid rise in plasma fibrinogen concentration. The RETIC study comparing FFP with fibrinogen and prothrombin complex concentrates was terminated early for safety reasons because of the high proportion of patients in the comparator arm (FFP group) who required rescue for correction of trauma induced coagulopathy (TIC) compared with those in the fibrinogen concentrate group. The European prehospital trial FIinTIC (NCT01475344) found physicians were able to administer fibrinogen concentrate in the field with an improved coagulation profile on arrival at hospital [33]. Finally, both FEISTY (NCT02745041) [34] and ProoF-iTH (NCT02344069) [35] have completed recruitment with results awaited. Of note, a 120-patient RCT using targeted fibrinogen concentrate infusion vs placebo in high-risk cardiac surgery patients found no difference in intraoperative blood loss [36]. Interestingly, more adverse events within 30 days were observed in the fibrinogen group (including stroke and transient ischemic attack, MI, and deaths) although there were some imbalances between groups. The relevance of this finding to trauma is unclear as supranormal plasma levels of fibrinogen after supplementation in observational studies, and small clinical trials do not appear to be associated with any adverse outcomes [32, 37].

In summary, hypofibrinogenemia is common in the retrospective reports detailed above and is

associated with worse survival. The administration of a high ratio of fibrinogen to each RBC unit is associated with improved outcomes, with some suggestive evidence of clinical efficacy from small prospective studies of cryoprecipitate and fibrinogen concentrate. The strongest evidence to support the use of cryoprecipitate, albeit non-randomized data, comes from the MATTERs II Study. Before adoption of early and aggressive use of fibrinogen replacement, the results of CRYOSTAT-2 are required to assess the impact of this change in transfusion priorities on survival from major bleeding in trauma.

Cryoprecipitate vs Fibrinogen Concentrate: Which Is Best?

Of the two therapeutic options available for fibrinogen replacement, only cryoprecipitate is recommended for management of acquired bleeding disorders in the United Kingdom, North America, and Australia. However, increasing numbers of blood transfusion services particularly in Europe are replacing cryoprecipitate with fibrinogen concentrate. A systematic review comparing the efficacy of cryoprecipitate and fibrinogen concentrate found very little high-quality data to draw meaningful conclusions [38]. Across the four studies that were included in this review, there was no difference in fibrinogen increment, transfusion requirement, thromboembolic events, or bleeding. In vitro and ex vivo work have shown that these two blood products lead to similar improvements in coagulopathy during trauma hemorrhage and the effects are dependent on fibrinogen concentration rather than formulation [4]. Interestingly, cryoprecipitate in one study did appear to provide additional benefit on the reversal of fibrinolysis compared to fibrinogen concentrate [39]. In citrate blood specimens from healthy subjects spiked with tissue plasminogen activator (t-PA) and subsequently supplemented with cryoprecipitate, FXIII, fibrinogen concentrate, and E-aminocaproic acid (EACA), hyperfibrinolysis was assessed using the clot lysis index at 60 min on ROTEM. Fibrinolysis was attenuated after cryoprecipitate supplementation compared to t-PA alone and compared to FXIII and fibrinogen concentrate. The additional plasma components in cryoprecipitate (VWF, FVIII, FXIII, and fibronectin) not present in fibrinogen concentrate may therefore have a role in mitigating hyperfibrinolysis although this has not been tested in the in vivo models or observed in human subjects.

In a number of European countries, fibrinogen concentrate is the main product for replacing fibrinogen due to its increased viral safety profile, standardized concentration of fibrinogen, freedom from blood incompatibility issues, and the benefits of accessing a lyophilized product quickly. However, it is important to emphasize that the two products are different not only on their contents but also on cost. Several factors contribute to a calculation of the cost of not only the product itself but also processing, storage, administration, geographical location, and type of product used with some reporting the unit cost of cryoprecipitate to be one-quarter of the cost per gram of fibrinogen concentrate [40]. An economic evaluation from the United States confirmed that even after cryoprecipitate wastage, fibrinogen concentrate is at least twice as expensive [41].

No study has yet been of sufficient size to assess the comparative clinical efficacy of the two formulations in trauma although results are awaited from the larger FEISTY trial (NCT02745041) in trauma with time to fibrinogen supplementation as the primary end point. Interestingly in the United Kingdom-based EFIT-1 trial [32], the median time to administration of fibrinogen concentrate was 40 min and therefore comparable to timelines for thawing and delivering cryoprecipitate although this is likely to reflect a learning curve effect. FIBRES is a non-inferiority trial conducted in 11 hospitals in Canada that compared cryoprecipitate with fibrinogen concentrate in cardiac surgery [42]. In patients who develop clinically significant bleeding and hypofibrinogenemia after cardiopulmonary bypass, fibrinogen concentrate was found to be noninferior to cryoprecipitate with regard to the number of blood components transfused in a 24-hour period post bypass. How this translates to uncontrolled hemorrhage in trauma is unclear, and until conclusive evidence

is provided for survival benefit or cost savings, cryoprecipitate looks set to remain the primary source of fibrinogen therapy across the majority of trauma systems. However, the portability and ease of storage of a lyophilized product such as fibrinogen concentrate may be well suited to austere environments such combat casualty care or civilian EMS which have a prehospital physician capability of administering blood component therapy.

Currently, there exists clinical equipoise for which product is the most effective for replenishing fibrinogen stores, supporting hemostasis during traumatic coagulopathy and impacting survival. Aside from the possible outcome benefits of early fibrinogen replacement, there are risks to consider with the administration of any blood component as well as the potential thrombotic complications of all procoagulant therapies. Finally, there is the wider cost analysis which relates not only to the product itself but also to blood bank logistics, wastage, and administration.

Guidelines and Thresholds for Triggering Fibrinogen Replacement

Despite the lack of evidence from randomized controlled trials, guidelines have recommended replacement during hemorrhage if the fibrinogen level falls below 1.0 g/L. This recommendation is based on expert opinion and a single nonrandomized report of 36 patients [43]. This report detailed the bleeding outcomes of 36 patients transfused modified whole blood (RBCs in cryosupernatant) who had their fibrinogen levels measured for every 12 RBC units given. Four patients dropped their fibrinogen below 0.5 g/L, and all four were classified as having "nonsurgical" or coagulopathic bleeding. In addition, of the ten patients who had fibrinogen levels drop between 0.5 and 1.0 g/L, two had coagulopathic bleeding. Based on this very small report, numerous guidelines recommended maintaining the fibrinogen level in excess of 1.0 g/L. More recently, the use of viscoelastic coagulation tests, e.g., ROTEM and TEG, have been recommended in the UK [44] and European guidelines [45] as a valid assessment of fibrinogen concentration to guide fibrinogen replacement.

The British Committee for Standards in Hematology recommends fibrinogen replacement if the level drops below 1.5 g/L in their 2015 guidelines and to consider empiric cryoprecipitate after the first four units of RBC and FFP [16]. In comparison, the American Society of Anesthesiologists (ASA) for perioperative blood transfusion recommends a lower replacement threshold of 0.8–1.0 g/L [46]. The most recent European Guidelines on Bleeding in Trauma (2019) are more specific and recommend treatment with fibrinogen concentrate or cryoprecipitate if major bleeding is accompanied by hypofibrinogenemia defined as either viscoelastic signs (ROTEM, TEG) of a functional fibrinogen deficit or a plasma fibrinogen level $\leq$1.5 g/L [45]. They suggest an initial fibrinogen supplementation dose of 3–4 g either with cryoprecipitate (15–20 single-donor units) or with FC (3–4 g), and repeated doses should be guided by viscoelastic monitoring and laboratory assays. It remains unclear what an appropriate replacement threshold should be in a bleeding trauma patient, but maintaining a plasma fibrinogen level or functional assay within the normal range (>1.5 g/L) during bleeding appears a reasonable target for triggering fibrinogen replacement treatment. Empiric therapy of cryoprecipitate during MHP remains an area of debate, but it is the first clotting factor to fall to critical levels and, therefore, early replacement in the initial transfusion packs may be appropriate. Some centers that routinely utilize fibrinogen concentrate and ROTEM or TEG have moved to goal-directed algorithms that prioritize replacement for functional deficits in fibrinogen although to date none have been validated widely [47].

Dosage and Infusion

ABO nonidentical cryoprecipitate is acceptable as long as it has low titer "anti-A" and "anti-B" activity. However, group O cryoprecipitate should only be given to group O patients to avoid

the potential risk of hemolysis. Specifications for national transfusion services vary, but each pool of cryoprecipitate contains 1.67 ± 0.27 g of fibrinogen and, therefore, a standard adult dose of 2 pools (10 single units) would provide approximately 3.3–3.6 g of fibrinogen. Once thawed, cryoprecipitate must be kept at room temperature and used within 4 h. The product is infused through a 170–260 μm filter, similar to all other blood components. The dose of cryoprecipitate is often listed at 1 unit per 5–10 kg of body weight, although no dosing studies have been performed to support this dosing recommendation. In a large Canadian study, the use of cryoprecipitate was evaluated in 1004 patients [48] of whom 31% were trauma patients. A median dose of 8.7 units resulted in a fibrinogen rise of 0.55 g/L (0.83–1.38 g/L) for patients who received only cryoprecipitate between the two measurements ($n = 83$ events) or 0.07 g/L rise per unit of cryoprecipitate. In both ex vivo data [4] and the CRYOSTAT-1 trial [32], a 6 g dose of fibrinogen, as cryoprecipitate or fibrinogen concentrate, results in significant increases in ROTEM clot strength values with a signal to improved survival, suggestive of clinical efficacy.

Adverse Reactions

In terms of the risk of viral transmission per unit transfused, each unit of cryoprecipitate has a similar risk to each RBC unit transfused, although there is a much larger donor exposure per pool (usually ten donors). Fibrinogen concentrate is made from pooled plasma utilizing many thousands of donations, thus increasing donor number exposure for any recipients of this treatment which may be an important consideration for emerging pathogens. However, the concern regarding the risk of viral and other infectious agents was one of the drivers prompting many European countries to switch from cryoprecipitate to fibrinogen concentrates.

Data from the UK hemovigilance scheme, Serious Hazards of Transfusion (SHOT) (1996–2014) found no cryoprecipitate-related cases of viral transmission were reported [49] until the 2015 SHOT report where one case of cryoprecipitate implicated HEV transmission was recorded. For comparison, over this same time period, there were five cases of viral transmission with FFP (three HEV, one HIV, and one HBV). The adverse events reported from cryoprecipitate include very rare reports of acute hemolysis from anti-A/B, minor febrile and allergic reactions, respiratory distress, and thrombosis. Readers interested in reviewing these rare risks are directed to a review of cryoprecipitate [50]. In general, there are few transfusion-related adverse events attributable to cryoprecipitate, and in the UK SHOT report for 2014, only 1 of the 43 acute transfusion reactions was attributed to cryoprecipitate, and cryoprecipitate was issued over 43,150 times that year [49].

High fibrinogen levels are a risk factor for thrombosis, and both trauma and shock are risk factors for thrombotic events. Few data exist on risks of cryoprecipitate, but results from a hemovigilance scheme in Quebec reported 6.57 events per 10,000 units of cryoprecipitate (from a total of 13,692 units administered) across all patient groups. In post-surveillance monitoring, one thrombotic event per 23,300 doses of fibrinogen concentrate was reported from data covering a 27-year period across all patient groups with fibrinogen deficiency [51]. In patients who have received fibrinogen concentrate therapy for major bleeding, no concerns have been raised around thrombotic risk or safety [52].

Ongoing Trials

CRYOSTAT-2 (ISRCTN 14998314) is the first RCT to evaluate whether early administration of high-dose cryoprecipitate, in addition to standard major hemorrhage therapy, improves survival from traumatic bleeding. The clinical trial will be the largest in trauma transfusion ever conducted and is a unique international collaboration between the Centre for Trauma Sciences (Queen Mary, University of London), UK NHS Blood and Transplant Clinical Trials Unit, and

University of Texas, Houston, to enable recruitment of all 25 UK major trauma centers and a number of Level 1 centers in the United States. The primary end point is all-cause mortality at 28 days with secondary end points evaluating early deaths (6 and 24 h), late deaths (6 and 12 months), transfusion requirements, hospital resource use, quality-of-life measures, safety outcomes for arterial and venous thrombotic events, and discharge destination. The trial intervention consists of early fibrinogen supplementation in the form of three pools of cryoprecipitate, providing approximately 6 g of fibrinogen, within 90 min of admission in addition to standard (local) MHP. The comparator arm is the standard (local) MHP alone. Overall trial recruitment will be 1568 patients and is powered to detect a mortality difference of 7% between the two groups assuming a baseline mortality of 26%. Dosing of fibrinogen for the trial was chosen using results from ex vivo coagulation testing and CRYOSTAT-1 pilot data with the aim of increasing blood fibrinogen levels by 1–1.5 g/L and to maintain levels above 2 g/L during bleeding [4].

Future Developments

It is likely that in the near future, the speed of delivering cryoprecipitate will be improved, and one option could be extending the shelf life of thawed cryoprecipitate (like FFP), making it readily available. Alternatively, some pharmaceutical companies are already looking at developing freeze-dry cryoprecipitate that can be made available quickly to patients with the preservation of the additional coagulation factors normally found in the standard product. Blood services have already begun to investigate extending post-thaw shelf life, both to 24 h [53] and 72 h [54], and no significant reductions in fibrinogen, FVIII, FXIII, or VWF were found after storage at 20–24 °C at either time point and preserved hemostatic function as measured by ROTEM parameters at 72 h. Extended thaw times with cryoprecipitate located in ED or carried by prehospital care providers may in theory make it even quicker than concentrate administration and thus provide an even more attractive therapeutic for major hemorrhage.

Summary

Fibrinogen is a key part of the hemostatic system. Despite the availability of cryoprecipitate since 1964, its role in the management of the bleeding trauma patient remains unclear. Hypofibrinogenemia is common in adult (less than <1.5 g/L in 14%, <1.0 g/L in 5%, and <0.5 g/L in 3%) and pediatric trauma patients (52% <2.0 g/L, 20% <1.0 g/L, and 11% undetectable). A preserved fibrinogen level on arrival to the hospital is associated with substantially better outcomes (odds of death reduced by 0.22 during the first 28 days for every 1 g/L rise in baseline fibrinogen). Worldwide, approximately 10% of cryoprecipitate distributed to hospitals is transfused to trauma patients. Adult trauma patients in the United States and the United Kingdom on average receive a dose of approximately 10 units of cryoprecipitate at about 3 h after arrival to hospital (at about the eighth unit of red cells), yielding a rise in fibrinogen level of 0.5–0.9 g/L posttransfusion. Several retrospective studies and small prospective trials in adult and pediatric, civilian and military, trauma settings suggest that more aggressive use of fibrinogen replacement may improve outcomes. It is unclear what should be the appropriate dose, threshold for administration, or hemostatic target level. Studies from the settings of cardiac surgery and postpartum hemorrhage suggest that the target of 1.0 g/L in some guidelines may be inadequate for some patients and that a higher target may be necessary (>1.5–2.0 g/L). While we await the completion of the large CRYOSTAT-2 RCT, it is reasonable to administer cryoprecipitate if the fibrinogen is under 2 g/L and/or within the early transfusion packs if the patient has serious ongoing hemorrhage requiring activation of an MHP. A dose of 10 units or 4 g of fibrinogen concentrate (or 50 mg/kg of fibrinogen in pediatrics) is a reasonable starting dose for adult trauma patients, but

additional and ongoing replacement may be required as guided by the results of laboratory assays or point of care viscoelastic coagulation monitoring.

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Platelet Transfusion

23

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Objective of Chapter

To review the biology of platelets as a blood product, to provide the clinician with a practical guide for their optimal use, and to serve as a knowledge platform for those interested in platelet transfusion research. The use of whole blood as a platelet containing product for the use in bleeding patients is also reviewed.

The Evolving Role of Platelets in Trauma Care

Platelet (PLT) biology is deceptively complicated. From a morphological aspect, the elegant discoid shape of the resting platelet belies the intricate cytoskeleton and convoluted tubular systems that enable spectacular shape change upon activation. The evolution of platelet products and their use in transfusion is similarly convoluted and requires an immersion in history to understand. As described below, the current state of the art in platelet transfusion rests on surprisingly little robust clinical data, minimal efforts to tailor product characteristics to patient needs, and a regulatory framework that supports the inadequate status quo. Development of new platelet-containing products and optimal transfusion strategies for bleeding patients represent opportunities for major advances in the care of bleeding patients or those at high risk of bleeding.

The Origins and Evolution of Clinical Blood Banking and Platelet Component Use

The first description of platelets is generally credited to Alfred Donné, a nineteenth-century French public health physician and microscopist, who published his drawings in 1842 [1, 2]. Prat published a primitive methodology for platelet counting in 1905, which was much improved upon by Wright in 1910–1911 [3, 4]. A more accurate manual counting method was not developed until the 1950s [5]. Duke, working with

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Wright at the Massachusetts General Hospital in 1910, published a seminal paper that described the spontaneous bleeding tendency resulting from thrombocytopenia and a method for testing platelet function that would become known as the Duke bleeding time (BT) [6]. In that paper, Duke showed that transfusion of fresh whole blood (WB) could raise a patient's platelet count and reduce both the BT and clinical bleeding, as evidenced by the resolution of epistaxis, melena, and other mucocutaneous bleeding. Based on the experience from the 30 cases presented in this paper and from experimental work on an unspecified number of dogs and rabbits, Duke concluded that a bleeding diathesis from thrombocytopenia was only likely when platelet counts dropped below 50,000/μL. Although his conclusions have not been thoroughly tested in the many clinical settings in which platelets are used, this transfusion threshold continues to inform guidelines to this day.

As the light faded on the last days of World War II (WWII), a new era in medicine began. Building on the experience of pioneers of the field in WWI, US and British physicians in WWII conclusively established the vital importance of whole blood transfusion as a life-sustaining therapy for traumatic hemorrhage, providing not only oxygen delivery but also hemostatic potential. Red cells, platelets, and plasma were delivered as fresh whole blood from a "walking blood bank," stored whole blood or freeze-dried plasma in WWII [7, 8]. Edwin Cohn pioneered the concept of blood fractionation in his work on plasma protein separation and the introduction of albumin as a resuscitative product in the 1940s [9, 10]. The therapeutic effects of donated blood would then be multiplied across the spectrum of human disease by the advances that led to modern blood banking and the adoption of component therapy, beginning in earnest in the 1950s with the early developments in blood apheresis. This revolution promised to meet the demands of the "right product, right patient, right time" [11, 12]. Researchers shared a vision of an era in which whole blood would no longer be required to meet the needs of patients with a critical deficiency in a single blood cell line.

The major impetus for the development of blood banking, in general, and platelet component transfusion, in particular, was the harnessing of cytotoxic chemotherapy and radiation in the treatment of cancer. These advances were preceded by observations in the aftermath of the 1943 US Navy ship bombings in Bari, Italy, that sailors exposed to alkylating mustard agents exhibited myelosuppressive effects, including thrombocytopenia. By the early 1950s, civilian and military research had converged to build the foundations of clinical oncology, and patients were experiencing the infectious and hemorrhagic complications of increasingly effective cancer care [13].

From its beginning, platelet transfusion was focused on treating thrombocytopenia, mostly in the context of bone marrow suppression caused by chemotherapy. Early studies in this area identified the difficulties in isolating and storing platelets without activating them, in addressing the immunogenicity of platelets, and the potential for alloimmunization and platelet refractoriness in chronically transfused patients [14]. Early key improvements included the transition from open to closed and glass to plastic storage systems that decreased risks of bacterial contamination and platelet activation during storage, respectively [15]. Through all of this work, the sine qua non of success in platelet transfusion was defined by the ability to raise platelet counts and maintain these gains for as long as possible, thus decreasing the risk of alloimmunization and platelet refractoriness in frequently transfused chemotherapy patients [16]. Indeed, "platelet viability" was defined in these terms, and platelet blood banking evolved along lines that optimized posttransfusion recovery and duration of platelet circulation or survival over several days, in vivo [17–19]. An early observation was that refrigeration of platelets or whole blood caused a drop in platelet count in the stored product and more rapid clearance of the stored platelets from circulation

over 2 days compared to fresh platelets, and as a result, the obvious benefits of refrigeration such as reduced metabolic stress and risk of bacterial growth were discounted [17, 20]. Room-temperature (RT) storage was adopted after studies showed increased in vivo recovery and survival ("viability") compared with refrigerated storage (4 °C) [20]. Generations of physicians have thus been misled by the confusion caused by this use of the term "viability" and the underlying assumption that because refrigerated platelets do not circulate as long as fresh platelets, they are therefore dead or nonfunctional [21, 22]. "Viability" when defined in terms of recovery and survival of stored platelets does not correlate with hemostatic efficacy or measures of metabolic function, and there have been very few studies that have rigorously studied platelet dose-response [23–25]. In fact, a more clinically relevant understanding of platelet function, encompassing hemostatic, trophic, and immune functions, might place greater weight on measures of platelet function that correspond to desired transfusion outcomes such as acute hemostasis [26].

An example of how the focus on "viability," or in vivo survival time, has obscured clinically relevant metrics of platelet product function can be found in the Korean War experience. Wounded soldiers in this conflict, as in WWII, were transfused cold-stored whole blood, which was widely assumed to contain nonviable platelets. Detailed studies of hemostasis in Korean War casualties, however, describe normal postoperative platelet counts despite massive transfusion (>20 pints) with cold-stored whole blood and only rare coagulopathic bleeding [27]. Such results belie the notion that cold-stored platelets are truly nonviable. These platelets were clearly able to support acute hemostasis during surgery and in the immediate postoperative period despite relatively accelerated clearance. The few cases of coagulopathic bleeding can be understood if one takes into consideration that at this time, as in WWII, blood was transfused cold, at its storage temperature (4 °C), to simplify administration and avoid the perceived risk of hemolysis during rewarming [28]. Massive transfusion patients of this era thus faced a major risk of hypothermia with resulting coagulopathic bleeding and, indeed, researchers at the time did not attribute occasional bleeding tendencies to a defect in stored platelets [27]. It was not until the mid-1960s that warming blood from refrigerator temperature to body temperature was identified as a critically important step in transfusion practice [29]. From WWII to the Vietnam conflict, blood transfusions were composed of mostly stored, but also fresh, whole blood. During this time, the Korean War experience seemed to confirm Duke's assertion that a platelet count of 50,000 was the critical level below which disorders of hemostasis would result, because the whole blood contained effective platelets able to participate in acute hemostasis, and platelet counts rarely fell below that threshold [27, 30]. During the Vietnam experience, the transfusion of 8- to 20-day-old acid citrate dextrose (ACD) preserved whole blood was thought to cause dilutional thrombocytopenia due to nonviable platelets in the blood with ensuing coagulopathy after 20 units and a platelet count of 50,000. In reality, a close examination of the data reveals that posttransfusion platelet counts exceeded predicted values by a significant margin, and platelet counts dropped to critical levels only after transfusion of about 30 units with simultaneous large-volume crystalloid resuscitation [31]. It is not surprising that coagulation disorders encountered under such circumstances were corrected with warm fresh whole blood, but it does explain why the surgical community was lulled into thinking that platelets mattered only when they were severely depleted—the condition was rarely encountered in the age of whole blood use [32]. As a result, the importance of platelets in the treatment of surgical bleeding and trauma went unrecognized, and research in this field was stifled until brought to the fore by the difficulty in supplying platelet components during the wars in Iraq and Afghanistan in the twenty-first century.

Clinical Evaluation of In Vivo Platelets and Platelet Components

The evolution of assays to assess the clinical efficacy of platelet transfusion is a metaphor for this inability to appreciate its vital role in reestablishing hemostatic function in trauma patients with hemorrhagic shock. Between 1910 and the late 1980s, simple platelet counts and Duke's BT method were the only assays routinely used. The latter, which was modified from an earlobe incision to one on the underside of the forearm while inflating a sphygmomanometer to 40 mmHg (the Ivy BT), was frequently unreliable as a platelet function test, as results were dependent on skin structure, blood vessel structure and integrity, hematocrit, temperature, fibrinogen, and Factor VIII (FVIII) and von Willebrand Factor (vWF) levels, as well as platelet count, size, and function [33–35]. Over the past 30 years, BT has been abandoned due to its imprecision, poor reproducibility, and discomfort for the patient. Platelet aggregometry, which measures platelet aggregation response to physiologic agonists like thrombin, collagen, and adenosine diphosphate (ADP), was developed in the 1960s and eventually became a "gold standard" but was never widely adopted in clinical practice, particularly in surgical settings, because the relative technical complexity of performing and interpreting the assay was never addressed by manufacturers due to the lack of emphasis on platelet function [33, 36]. Similarly, flow cytometry can be used to characterize platelet function, but this method, like aggregometry, has mostly been applied in research settings [37]. Unlike in the field of trauma, cardiologists and cardiovascular surgeons were much more aware of platelet effects in acute coronary syndromes; thus, modifications of aggregometry and viscoelastic testing (thromboelastography, TEG; rotational thromboelastometry, ROTEM) have been developed to support the management of platelet inhibition therapy [38]. The aforementioned technologies predict bleeding risk and platelet transfusion in surgery, but, in keeping with the general disregard of platelet efficacy, so far, are not required by regulatory guidelines in the evaluation of platelet products for the treatment of hemorrhage [39, 40]. In short, reliance on BT and failure to adopt other standards suggests that, until recently, measurement of platelet function after transfusion has not been a priority for trauma surgeons and critical care physicians, platelet transfusion researchers, or regulatory agencies. In summary, the focus of posttransfusion evaluation has been on raising platelet counts in chemotherapy patients, with the underlying assumption that platelets that circulate must be functional [41].

The relative lack of emphasis on platelet transfusion for treating surgical hemorrhage and trauma should be surprising, given the Vietnam War-era evidence demonstrating that platelet dysfunction plays a role in trauma-induced coagulopathy, but awareness of the data's import was not evident in the writing of the time [42]. As noted, cardiothoracic surgery has since become the exception, but even there, interest is narrowly focused on correcting bypass circuit-induced thrombocytopenia. Prior to the twenty-first century, the relatively few studies that examined platelets as part of trauma care and massive transfusion did record the development of thrombocytopenia and evidence of platelet dysfunction [43–45]. Unfortunately, these observations were dismissed as irrelevant due to the infrequency of clinically apparent "medical bleeding"—oozing, petechiae, and purpura, as commonly observed in severe hypoproliferative thrombocytopenia or consumptive conditions like disseminated intravascular coagulation (DIC) [43, 44]. In contrast, Counts et al. noted that non-platelet-containing products such as red blood cell (RBC) concentrates resulted in hemodilution during massive transfusion and provided one of the few studies to suggest a role for platelets in preventing dangerous thrombocytopenia [46]. Nevertheless, even Counts et al. narrowly advocated platelet transfusion for the treatment of an established dilutional thrombocytopenia and platelet dysfunction. Their critical role in primary hemostasis, known for much

of the twentieth century, of plugging holes in the vasculature, catalyzing the generation of thrombin to form the clot-stabilizing fibrin mesh, and then retracting the clot in concert with blood vessel vasoconstriction was little considered. Investigators did not appear to see the need to support these functions by proactively replacing the platelets lost to severely traumatic or surgical hemorrhage. The link between thrombocytopenia and microvascular bleeding in oncology patients was much discussed, while the larger role platelets play in overall hemostasis and the response to injury, whether surgical or traumatic, was largely ignored.

Beginning in the late 1960s, platelet-containing stored and fresh whole blood products gradually disappeared from most hospitals as component therapy became widely available and the economics of blood banking favored fractionation of whole blood [46]. The growing threat of transfusion-transmitted disease virtually eliminated walking blood banks by the early 1980s. Surgeons in this era were using modified whole blood (generally meaning that platelets and cryoprecipitate had been removed), RBC concentrates, and saline, with limited amounts of plasma and platelets [31]. When massive hemorrhage from penetrating injuries became more common during the drug wars of the 1980s and early 1990s, interest in trauma-induced coagulopathy resurfaced, and with it, an appreciation that surgical bleeding outcomes were adversely affected because, with whole blood largely unavailable, RBC-focused resuscitation created iatrogenic deficits in plasma and platelets [47–49].

Newer point-of-care tests to rapidly assess coagulation, evaluating functions such as clot contraction, are currently in development [50]. Platelet contraction is an energy-dependent biologic process reflective of hemostatic potential that also serves as an indicator for systemic metabolism [51]. The platelet contraction assay may be more successful at determining the efficacy of antiplatelet drugs or optimizing anticoagulation where antiplatelet therapy is indicated [50].

Transfusion Products for Trauma Care

It is important to recognize that the current state of platelet transfusion science is inadequate to guide optimal resuscitation therapy, even if data-driven guidelines covering timing and quantity were available. The regulatory framework currently in place throughout the world evolved with the development of platelet products optimized for recovery and survival, and does not adequately address the hemostatic, trophic, or immune functions of platelets. While the US Food and Drug Administration (FDA) issued a draft "guidance to industry" document on platelet function testing in 1999, it does not appear to have been formally adopted or enforced and thus current standards assume functional integrity based on morphological appearance and responses [41]. Perhaps, the most important innovation in platelet transfusion was the adoption of apheresis over the preparation of platelet concentrates from whole blood donations (pooled platelets) [52]. Use of apheresis units reduces donor exposure; simplifies leukoreduction and thus prevents complications such as nonhemolytic febrile transfusion reactions; and allows human leukocyte antigen (HLA)-type and cytomegalovirus (CMV) seropositivity matching. While these are genuine advantages in the setting of hematopoietic stem cell transplantation, use of apheresis platelets confers no advantage with respect to hemostatic function. The practice of RT storage (22–25 °C) of platelets thus persisted despite profound functional deficits and the increased risk of bacterial growth compared to refrigerated or frozen blood products [26]. As noted previously, the recognition that refrigeration leads to rapid clearance of stored (over 2 days) compared to fresh (over 7 days) platelets is well established. While circulation time is critical for some, however, most patients typically have normal megakaryocyte function and thus require functional platelets to support hemostasis during active bleeding, after which they are able to replace depleted stores. Refrigerated platelets may be the ideal product for this population,

because they have enhanced ability to participate in coagulation and thus may control bleeding more quickly [53]. In both platelet concentrate and whole blood studies, cold platelets perform better with regard to platelet aggregation (collagen, ADP), viscoelastic testing (TEG and ROTEM), and other functional measures compared to those stored at RT [21, 22, 26, 53–56]. The RT platelet storage lesion results in increased levels of soluble CD40 ligand (sCD40L), a potent immune-stimulatory molecule, which reflects platelet degranulation and is thus considered a marker of platelet functional loss. Stored RT platelets accumulate more sCD40L over time compared to those stored at 4 °C [21, 57], providing indirect evidence that cold-stored platelets may enhance hemostasis due to improved function. Life-threatening bleeding is typically controlled within 6 h of onset, during which time 4 °C refrigerated platelets circulate at high concentrations that are comparable to those stored at room temperature. On the other hand, room-temperature platelets rapidly accumulate functional defects during storage, losing their ability to aggregate in response to arachidonic acid, collagen, and ADP [21, 58–61]. Furthermore, Becker et al. and, in a separate study, Valeri showed that cold-stored platelets control bleeding more effectively in aspirin-treated volunteers compared to RT-stored [53, 62, 63]. Becker further demonstrated that cold-stored platelets are effective in treating bleeding thrombocytopenic patients. Conversely, RT-stored platelets may be ineffective in reversing the increased risk of surgical bleeding in patients taking aspirin and/or clopidogrel unless large doses (2 apheresis units) are given preoperatively [64–66]. Unfortunately, platelets stored at 4 °C were never compared to platelets stored at RT for the treatment of medical, surgical, or traumatic etiologies of hemorrhage and, indeed, no randomized controlled trials of platelet- versus non-platelet-containing transfusion strategies are published. Platelet refrigeration was abandoned by a transfusion medicine community focused on the needs of oncology patients who were thought to benefit from prophylactic transfusions of platelets with longer circulation times. Other approaches to platelet preservation, motivated mainly by the need to expand available inventories, were attempted. For example, cryopreserved platelets (CPP) frozen in the cryoprotectant, dimethyl sulfoxide (DMSO), were developed by Valeri as early as the 1970s, and Crowe pioneered the use of trehalose in platelet lyophilization [67, 68]. Since neither of these approaches yielded products that improved platelet counts in a sustained manner, their development stalled. Valeri conducted a small randomized controlled trial (RCT) comparing RT platelets to CPP in cardiothoracic surgery bypass-induced thrombocytopenia and showed that CPP were more efficacious than RT platelets and likely safe [69]. The available data thus indicate that 4 °C and CPP are superior to RT platelets in treating bleeding patients in some settings; nevertheless, comprehensive RCT evaluations of different platelet-containing products, namely, fresh whole blood (FWB), stored whole blood (SWB), RT-stored platelets, 4 °C-stored platelets, and CPP, are lacking.

The resulting shortages in platelet inventories ensure that platelet transfusion is largely reserved to treat the most severe thrombocytopenias, primarily in oncology patients. Ironically, three large, multicenter RCTs in North America and Europe have recently demonstrated that prophylactic platelet transfusion in the oncology setting is of questionable efficacy, and no dose of RT platelets was identified as effective in reducing blood loss [24, 70, 71]. The notable exception to this finding is prophylactic platelet transfusion for patients undergoing induction chemotherapy or allogeneic stem cell transplantation for acute myelogenous leukemia (AML). These patients suffer profound and long-lasting thrombocytopenia, as well as treatment-related toxicity. Prophylactic platelet transfusion in this cohort reduces intracranial hemorrhage and mortality and should be continued, but many oncology patients currently receiving prophylactic transfusions do not fall into this category. As these data are incorporated into current guidelines, the proportion of transfusions for prophylactically maintaining an arbitrary platelet count is likely to decrease, and therapeutic transfusion for severe, acute hemorrhage may become the most common indication for platelet use.

Investigators in Bergen, Norway, have recently completed a pilot RCT of 4 °C platelets stored in platelet additive solution (PAS) versus room-temperature-stored platelets in plasma. At the time of this writing, the results of the above trial have been presented, but not published. The results indicate it is feasible to perform an RCT and there is potential for reduced bleeding with the use of platelets stored cold for up to 14 days. There was also no increase in adverse events in the cold-stored group. These findings have supported the development of a multicenter RCT that will use an adaptive trial design to determine if cold-stored platelets reduce bleeding in cardiac surgery patients compared to the use of room-temperature-stored platelets. The Chilled Platelet Study (CHIPS) intends to enroll up to 1000 patients and is planned to start in late 2020.

These recent results show that, despite over six decades of research on platelet transfusion, the use of RT platelets for bleeding patients is not optimal; they suffer major functional deficiencies and may pose unnecessary risks of bacterial contamination. The neglect of platelet function and insistence on RT storage to maximize circulation time restrict shelf life to 5 days in order to reduce risks of bacterial growth. The current rate of septic transfusion reactions remains highest in platelet transfusions despite numerous safety measures. In response, additional guidance has been issued by the FDA regarding bacterial testing of platelets stored at room temperature (21 CFR 606.145a). The FDA will now require additional safety measures beyond a primary bacterial culture for room-temperature-stored platelets. Platelets collected by apheresis or prestorage whole blood-derived pools may be pathogen reduced or undergo either secondary culture or secondary rapid testing after the primary culture. In addition, revisions were made to clarify recommendations related to dating periods, labeling, inventory management, and culture incubation periods.

Cold storage of platelets has the potential to overcome many of the abovementioned shortcomings associated with RT platelet storage. Currently, the FDA allows for cold storage of platelets at 1–6 °C without agitation for up to 3 days for trauma patients (21 CFR 640.24 and 640.25). In 2015, the Mayo Clinic successfully implemented the use of such 3-day platelets for trauma patients. The military, after significant research and data collection, submitted a variance request to the FDA. This variance was approved by the FDA in August 2019 allowing storage of apheresis platelets at 1–6 °C for up to 14 days without agitation [72]. The cold-stored platelet products will be used to treat actively bleeding patients when conventional platelet products are not available or their use is not practical. This variance may enable further reemergence of cold-stored platelet use by more facilities in the very near future and serve as a critical first step in delivering platelets to the battlefield in order to decrease life-threatening bleeding due to trauma.

Evidence from the Wars in Iraq, Afghanistan, and Beyond: A New Direction for Platelet Transfusion Medicine?

The relative unavailability of platelets and the iatrogenic deficits in plasma and platelets noted above essentially describe the state of affairs when the United States embarked on the recent conflicts in Iraq and Afghanistan, beginning in 2001. Civil unrest and insurgent activity heightened the conflict in 2004–2005, and the casualty rate rose, giving military physicians sufficient experience to realize that resuscitation with RBC concentrates and crystalloid was ineffective in managing combat trauma-related massive hemorrhage. Platelets and plasma were needed, but platelet concentrates were not available because of the 5-day shelf life and requirement to only use platelets collected in the United States. The lack of platelet availability was one reason that prompted emergency warm fresh whole blood collections as a platelet-containing alternative, and in dramatic fashion, a new generation of physicians rediscovered the importance of providing whole blood functionality to the shocked, hemorrhaging patient [73, 74]. The military transfusion medicine community resisted this return to warm

fresh whole blood due to concerns regarding infectious disease risk and streamlined transport systems to provide timely delivery of plasma, platelets, and cryoprecipitate which were reconstituted into a whole blood-like cocktail [75, 76]. Retrospective analyses of this experience revealed that fresh whole blood was at least as effective if not more so than component therapy and that the effectiveness of component therapy was dramatically enhanced when plasma and platelets were included early to reapproximate whole blood function at the outset of resuscitation [73, 77–79]. These observations gave rise to the "1:1:1" ratio-based massive transfusion protocol, which was subsequently validated in large retrospective civilian trauma studies. Recently, investigators funded by the US Department of Defense and the National Institutes of Health conducted the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR trial) trial, a large prospective randomized trial comparing a 1:1:1 ratio versus 1:1:2 in which the latter group received fewer platelets given later in resuscitation [80]. The study was limited by the similarity of the treatment arms due to ethical considerations, which may be the reason that there was no difference in the primary outcome of all-cause mortality. Further analysis, however, demonstrated a difference in secondary outcomes, including superior early hemostasis in the 1:1:1 group and fewer deaths due to exsanguination by 24 h. These results provided higher-quality evidence to support equal ratio component use, but importantly, the PROPPR trial could not differentiate between platelet and plasma effects and was not designed to evaluate platelet transfusion efficacy. The intriguing finding of Stansbury et al. that trauma mortality outcomes are directly proportional to admission platelet count, including counts well above the 50,000 platelet per microliter accepted minimum threshold [81], does suggest that some of the 1:1:1 benefit is likely due to platelet effects and is corroborated by retrospective data from the recent conflicts in Southwest Asia [77–79, 82]. Stanbury also noted that as admission platelet count increased across the entire normal range, outcomes improved [81]. The link between platelets and outcome has been further reinforced by others who described the onset of a trauma-induced platelet dysfunction occurring prior to iatrogenic hemodilution and other interventions and illustrated that surgical hemostasis is a function of platelet activity as well as count [43, 44, 83–85]. After more than 60 years of platelet transfusion history, this poorly understood anucleate blood cell has attained recognition as a critical component of transfusion therapy in trauma and surgical resuscitation.

Transfusion-Related Platelet Biology

Non-hemostatic Effects of Platelets

The vascular space is home to many different cell types with various effector functions, platelets not excluded, as well as four proteolytic cascade systems. While traditionally thought solely to participate in hemostasis and thrombosis, platelets have been shown to play important roles in modulating inflammation and immunity through interactions with leukocytes, the vascular endothelium [86], and the complement system [87]. During homeostasis, endothelial cells secrete various factors (e.g., nitric oxide, prostacyclins) which promote platelet quiescence and maintain vascular flow. Upon biological, chemical, or mechanical insult, endothelial cells can become injured or activated. In the case of the former, damage to the endothelium results in the exposure of the subendothelial matrix, allowing for platelet binding to collagen and tissue factor, thereby initiating hemostasis – the traditional thrombocyte role. Alternatively, during activation in response to inflammatory signals, endothelial cells release vWF and platelet agonists, such as thromboxane; they also mobilize P-selectin to the endothelial surface. These factors promote platelet adherence and binding to the endothelium and subsequent platelet activation, resulting in platelet secretion and production of multiple cytokines and chemokines with various pro-inflammatory and chemotactic effects, including IL1-b, IL-6, IL-8, IL-12,

CCL2 (MCP-1), CCL4 (MIP-1β), and TNFα [88]. Moreover, activated platelets not only secrete chemokines to recruit leukocytes but also serve as additional platforms for leukocyte binding and engagement. Platelets express adhesion molecules, such as CD40L, P-selectin, PECAM-1, and GPIbα, which then directly interact with their cognate receptors expressed by leukocytes (CD40, PSGL-1, PECAM-1, and Mac-1 (integrin αMβ2 or CD11b/CD18)). Activated platelets thus play an important role in potentiation of the inflammatory process via leukocyte recruitment, activation, and endothelial cell activation, all at the site of vascular injury.

While platelets have been shown to modulate both innate and adaptive immune functions [89, 90], how platelet transfusion alters these known immune-modulating functions is not quite so clear. With respect to complement-platelet interactions, the platelet itself provides a surface for local complement activation, inducing not only a procoagulant platelet [91] but also the release of anaphylatoxins C3a and C5a, which are potent chemoattractants and stimulators of leukocytes [92]. Endogenous complement levels can exacerbate refractoriness in allo-sensitized patients receiving platelet transfusions through HLA antibody-mediated complement activation [93]. Of note, complement activation was shown to increase over time during platelet storage [94]. From work in other fields, complement activation has also been shown to modulate T-cell effector functions [95]. More detailed studies are needed to determine if platelet transfusion-mediated complement activation can play a role in altering lymphocyte function.

Limited work exists chronicling the mechanistic effects of platelet transfusion on endothelial function and permeability. Baimukanova et al. reported that platelet storage duration, both at 4 and 22 °C, led to decreases in platelet-mediated activation and the ability to reduce the permeability of endothelial monolayers [96, 97]. They also showed that both 4- and 22-°C- stored platelets reduced vascular leak but that 22-°C-stored platelets had superior efficacy in vivo in a mouse model of the vascular leak [97]. Lastly, platelet-derived extracellular vesicles were shown to be as efficacious as platelets themselves in restoring vascular permeability in a murine model. While these studies start to elucidate the interactions between platelet transfusion and endothelial function, much work remains to be done.

Platelet Storage Lesion

The platelet storage lesion is defined as deleterious alterations in platelet quality from the time of collection until transfusion. Numerous complex factors contribute to the storage lesion and lead to changes in platelet structure, biochemical processes, and metabolic parameters due to collection technique, preparation, and storage conditions [98]. Factors that influence platelet quality include time, temperature, pH, lactate levels, suspension medias such as platelet additive solutions, the presence or absence of leukocytes, storage bag materials, and shear forces [58, 59, 98, 99]. These conditions affect the biology of stored platelets and modify their functional responses and circulating times. Understanding the metabolic, structural, biochemical, and functional changes will likely permit future improvements in the storage and transfusion of platelet products.

Metabolic Indicators

Proper oxygenation of stored platelet concentrates (PC) is important as hypoxia induces a switch to anaerobic glycolysis leading to lactic acid accumulation, reduced pH, and platelet impairment [58]. Studies demonstrating that PCs have an oxygen consumption rate of approximately 1.10 nmol/min/10^9 following 24 h at room temperature led to the recognition of the importance of oxygen exchange during storage and to the development of novel bag materials and plasticizers to optimize gas exchange [100]. In the presence of adequate oxygen, PC bicarbonate levels drop, and glucose consumption to lactate production occurs at a rate of approximately 1:2 moles, respectively [100].

Identification of the changes in platelet metabolic parameters during storage thus led to strategies for preserving and supporting the metabolic rate, such as refrigeration or the formulation of platelet additive solutions [59, 101]. As refrigeration slows metabolic rate, glucose consumption and lactate production decrease, whereas changes in bicarbonate levels and pH are minimized compared to 22 °C controls [21]. While the benefits associated with reduced metabolic demand are considerable, these come at the cost of a progressive drop in platelet count over time, suggesting that aggregate formation, adhesion, or cell death might be occurring in PC stored at 4 °C [21]. Furthermore, cold-stored platelets have reduced circulation times which may not be ideal for prophylactic platelet transfusion to treat thrombocytopenia in the setting of [20, 102].

The decision to store platelets at 22 °C for clinical use led to the development of platelet additive solutions (PAS) to address adverse effects on energy metabolism and to minimize platelet activation during storage [101]. All PAS have some basic components in common: sodium chloride (NaCl), a fuel source such as Na acetate or glucose, and Na citrate as an anticoagulant. Proprietary versions differ in composition with components such as sodium bicarbonate ($NaHCO_3$) or phosphate (PO_4^{3-}) to aid in buffering and/or magnesium chloride (MgCl) and potassium chloride (KCl) to limit platelet activation [103]. Despite continued efforts to optimize the storage environment, studies have yet to demonstrate that PAS confer sufficiently substantial improvements in maintaining platelet viability that would justify the added expense compared to plasma storage [104, 105].

Severe trauma and hemorrhage significantly alter platelet aggregatory function to agonists such as ADP, thrombin, and collagen [106–108] and subsequently reduce clot strength [109]. While this platelet dysfunction is likely a multifactorial response, recent evidence suggests that stimulation of adenylate cyclase causes a rise in an intracellular second messenger associated with inhibitory signaling; there is also a drop in high-energy phosphates, ATP and GTP [85], suggesting metabolic exhaustion of platelets.

Structural Changes

Platelets circulate in an inactive discoid shape and, upon stimulation, undergo responses including shape change, filopodia and lamellipodia formation, granule secretion, and aggregation. Platelets in the resting state have a macroscopic swirling appearance when a manual "swirling test" is performed, which, in room-temperature-stored samples, correlates well with the preservation of the discoid shape and in vivo circulation after transfusion [110]. Studies examining the cytoskeletal properties of platelets following chilling or activation identified the importance of the circumferential band of microtubules in supporting and maintaining the discoid shape [111, 112]. Once platelets are chilled below 15 °C, they undergo cold-induced shape change, which is characterized by depolymerization and collapse of the microtubule ring, increased cytosolic calcium levels, and actin assembly [111, 113–116]. As the temperature drops, platelets lose their swirling phenotype associated with discoid shape and, upon transfusion, are cleared from circulation within 2 days, compared to 4–5 days if stored at room temperature [20]. Reports indicate that brief periods of rewarming prevent or reverse the cytoskeletal changes if the exposure to the cold is less than 18 h [117]. Temperature cycling has been observed to improve microtubule reassembly, preserve platelet shape, improve aggregation responses, and increase circulation times compared to platelets continuously maintained in the cold [117, 118]. These results require confirmation, and thermocycled platelets have not yet been assessed in bleeding patients. Further research is required to confirm the clinical feasibility and utility of thermocycled platelets.

Following severe injury and hemorrhagic shock, platelets undergo morphological transformation into a highly procoagulant balloon shape [119, 120]. Severe trauma and hemorrhage lead to the release of histone H4 into the circulation, causing a sustained rise in platelet intracellular calcium, and driving platelet balloon formation [121]. Balloon formation has also been reported to occur in the in vitro setting in response to high concentrations of collagen and thrombin [122].

Platelet ballooning is considered to be a pivotal trait of platelets in hemostasis as it maximizes the surface area available for the assembly of procoagulant enzyme complexes.

Activation Markers and Granule Contents

Platelet granule secretion is important for the amplification of aggregation, recruitment of leukocytes, and vascular remodeling. P-selectin, an adhesion molecule found in the platelet α-granules, is rapidly expressed on the plasma membrane following activation [123]. Platelet storage studies indicate that P-selectin expression increases with time at room temperature and to an even greater degree when stored at 4 °C [21, 124, 125]. Additionally, α-granule constituents such as β-thromboglobulin and platelet factor 4 increase over time in the storage media, indicating that partial degranulation has occurred [126–128]. While P-selectin is a sensitive marker for platelet activation, it is unlikely to have a significant role in clearance and may be an unreliable predictor of posttransfusion platelet function [127, 129–132].

Microparticles/Phosphatidylserine Exposure

Phosphatidylserine (PS) is an anionic phospholipid expressed on the inner leaflet of the plasma membrane and is externalized after necrotic or apoptotic pathways are triggered in platelets [122]. The exposure of PS on the platelet surface helps support primary hemostasis by promoting thrombin generation through the assembly of the coagulation factors activated factor five (FVa) and activated factor ten (FXa) [133]. The constant metabolic demand during platelet storage is associated with the loss of mitochondrial membrane potential and PS exposure [134, 135]. Many of the necrotic and apoptotic features such as membrane fragmentation, cytoskeletal disruption, caspase activation, PS exposure, and microparticle (MP) formation have been observed following platelet storage [59, 122]. The clinical relevance of transfusing products containing MP and PS warrants further investigation. While platelet-derived MP may aid in the hemostatic process by accumulating at the site of injury and supporting fibrin formation, transfusion of procoagulant MP may enhance or promote systemic coagulation in susceptible patient populations [136–138], but the clinical significance of these functions remains to be fully defined.

Surface Receptors

Glycoprotein Ib-IX-V (GPIb-IX-V) mediates primary hemostasis through its interaction with activated von Willebrand factor and plays an essential role in clearing platelets from circulation [139]. Storage studies demonstrated that a GPIb-IX-V subunit, GPIbα, is shed from the platelet surface in response to the ADAM metalloproteinase domain 17 (ADAM17), also known as TNF-α-converting enzyme (TACE) [140, 141]. Inhibition of ADAM17 activity during room-temperature storage improves platelet survival and recovery, illustrating the importance of this receptor in platelet clearance [140, 142]. Other studies investigating the mechanism of cold-stored platelet clearance have identified two distinct modifications of GPIbα which mediate their removal from circulation [143]. Short-term chilling of platelets leads to the clustering of exposed β-N-acetylglucosamine (bGlcNAc) on GPIbα which is recognized by hepatic macrophage αM-lectin receptors [144]. The fact that bGlcNAc glycosylation with UDP galactose, a method to prevent hepatic recognition, fails to improve platelet clearance after prolonged storage at 4 °C suggests the existence of other platelet clearance mechanisms. Follow-up studies demonstrated that refrigeration causes the release of platelet sialidases, resulting in desialylation of GPIbα and thus platelets with reduced α2,3-linked sialic acid are removed from circulation by hepatic lectins, Ashwell-Morell receptors, through the binding of exposed βGal and GalNAc [145–147]. Furthermore, a recent finding suggests that clearance of desialylated platelets by

Ashwell-Morell receptors upregulates compensatory new platelet production by stimulating the expression of thrombopoietin [148].

Efficacy and Safety of Platelets Stored in Additive Solutions

Extensive research has been executed over the past several decades in order to understand how best to store blood components. Bag materials, storage solutions, and temperature have been major areas of focus for extending the shelf life, functionality, and in vivo recovery and survival times for blood components [21, 53, 103, 149]. Interestingly, differences in manufacturing procedures (collection platform, storage solution, or storage temperature) have yielded different in vitro characteristics and recovery and survival times [150, 151], demonstrating the importance of understanding the effects of each manufacturing change.

Platelet refrigeration results in partial cellular activation and therefore has raised concerns for increased thrombotic risk in the transfused recipient [21], although increasingly data have begun to suggest otherwise. In vitro results have shown that cold-stored platelets respond to physiologic inhibitors such as prostacyclin and nitric oxide [152]. Furthermore, the more rapid clearance of cold-stored platelets may enhance their safety by reducing exposure time. Therefore, there is a potentially lower risk for inducing thrombosis with cold-stored platelets.

Much of the research investigating PAS has been aimed at improving the quality of room temperature platelets [153]. Thus, there is most likely room for optimization of PAS for cold-stored platelets. Studies have demonstrated that platelets stored in plasma at 4 °C have a tendency to aggregate and that storage in PAS may mitigate these effects by dilution of plasma fibrinogen [154]. Reducing the citrate concentration in PAS has also shown to have favorable in vitro benefits [155]. Interestingly, no in vitro characteristics for stored platelets appear to accurately predict in vivo recovery and survival times. For example, the in vitro functional responses to stimuli for cold-stored platelets in plasma or PAS are comparable, but platelets stored in plasma elicited higher in vivo recovery compared to platelets stored in InterSol or Isoplate additive solutions [151]. Despite the lack of correlation between platelet in vitro characteristics and in vivo survival and recovery, it is worth mentioning that no data exist on what the radiolabeling procedure does directly to the surface characteristics and cellular function of radiolabeled platelets prior to transfusion for recovery and survival studies. Future optimization of PAS formulations for cold-stored platelets will require rigorous characterization to determent their impact on safety and efficacy.

Translating Platelet Research Findings into Artificial Platelet Products

As the preceding sections of this chapter make clear, platelet transfusion has had a complicated history, and currently, available platelet products leave much to be desired. As platelet research moves forward in earnest to fully describe structure, function, and diverse roles, creation of artificial platelet substitutes may become possible and would reduce the hazards of infectious disease transmission, alloimmunization, short shelf life, and complicated storage conditions. It is possible to envision that a standardized product could even allow for reliable dose-response hemostatic properties delivered with predictable pharmacokinetics, unlike current platelet concentrates whose properties are as individual as the donors themselves. Enterprising scientists have sought since the 1950s to develop artificial platelet products along two major lines of effort: platelet-derived hemostatic agents (PDHAs) and artificial hemostatic platelet substitutes [156]. The immune and trophic aspects have been less emphasized as these functions remain poorly understood, and because the challenges of merely delivering hemostatic function have been overwhelming. Nevertheless, applications of PDHAs in the promotion of wound healing are already being explored [157].

PDHAs are made by simple lyophilization; by platelet fixation and lyophilization; by using trehalose, a sugar made by desiccation-resistant organisms to stabilize platelet membranes prior to lyophilization; or by various attempts at generating thrombogenic platelet fragments including membrane preparations [158–160]. To varying degrees, these development efforts have succeeded in addressing the logistical challenges of providing platelet hemostatic activity to patients, mainly by increasing shelf life and simplifying storage. The most promising development projects to date have been Cellphire's Thrombosomes® (trehalose-loaded lyophilized platelets) and Entegrion's Stasix® products (glutaraldehyde-fixed, lyophilized platelets), funded by the US Department of Defense and the Department of Health and Human Services. The distinguishing in vivo features of products made by these approaches are rapid clearance, ability to support thrombin generation, variable adhesion to collagen, and reduced aggregation performance [161–163]. Animal model studies suggest safety and some ability to support hemostasis and reduce blood loss in thrombocytopenic models [164, 165]. These preclinical studies supported an investigational new drug (IND) filing and the first in human safety dose-escalation trial utilizing Thrombosomes collected from autologous sources via apheresis in normal subjects [166]. The trial identified that no serious adverse events (AEs) were related to the transfusion of autologous freeze-dried platelets. A future Phase II study will need to be conducted to determine further safety and efficacy of lyophilized platelets.

Artificial hemostatic platelet substitutes have proven more difficult to develop. Numerous fibrinogen- and vWF-binding platforms have been evaluated, including modified RBCs, albumin microparticles, liposomes, and hydrogels [167–169]. One of the more promising artificial hemostatic platelet substitutes is Haima Therapeutics's SynthoPlate™. The synthetic platelet surrogate utilizes a liposomal platform with surface decorations including von Willebrand factor-binding peptide, collagen-binding peptide, and an activated fibrinogen receptor-mimetic peptide [170, 171]. SynthoPlate preclinical data demonstrated a safety profile and showed signals of efficacy in a thrombocytopenic murine tail-transection model, murine liver injury model, and porcine femoral artery injury model [172–174]. While the in vitro and animal model results of some of these technologies are promising, advanced preclinical and clinical development will be required. The limited shelf life of currently approved platelet concentrates continues to create unmet needs and generate opportunities for further development of these technologies.

Clinical Platelet Transfusion Medicine

Transfusion Volume and Anticipated Change in Platelet Count

The measurement of platelet transfusion efficacy is complex due to the multiplicity of variables that can affect platelet participation in clot formation. Traditionally, simple platelet counts were used; however, this approach does not account for the volume of distribution, degree of thrombocytopenia, or the effect of chronic disease, among other factors. Platelet viability, pharmacologic agents, and medical conditions such as hypersplenism, hepatomegaly, and bone marrow transplantation can dramatically alter recovery [175–177].

The established research tool for determining platelet transfusion effect, a recovery and survival study, starts with the infusion of a radiolabeled platelet dose, followed by serial blood sampling to determine the percent of the original dose available for measurement (recovery), as well as the length of time, typically measured in days, over which the radiolabeled platelets are detectable (survival; see Fig. 23.1) [178]. Although higher values have been reported, approximately 66 ± 8% of the platelet dose is recovered on average immediately after transfusion in healthy subjects, a number that is slightly lower (approximately 60 ± 15%) in the setting of thrombocytopenia [179]. As investigators recognized that simply reporting percentages ignores

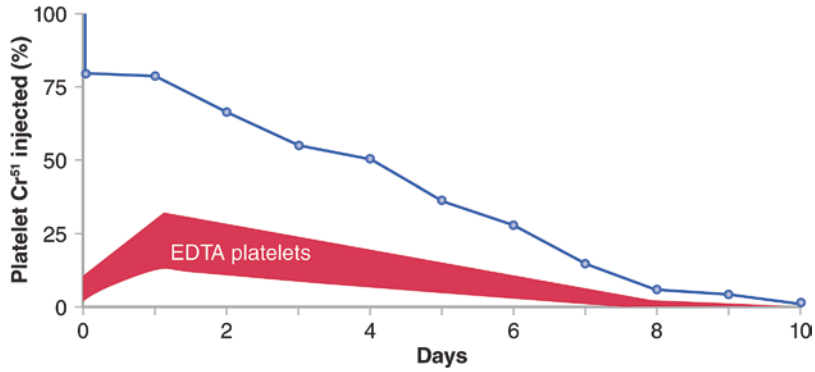


Fig. 23.1 Survival of autologous "citrate platelets" after transfusion to a normal subject demonstrates that approximately 75% of labeled platelets were recovered in the circulation immediately after being transfused. The *red area* denotes the range of blood platelet radioactivity after the injection of Cr51-labeled "EDTA platelets" on ten occasions in seven normal subjects, demonstrating that the maximum recovery was <40% with lower survival, due to EDTA-induced structural changes leading to more rapid clearance in the liver. (Modified from Ref. [178])

the influence of multiple patient and donor factors, methods such as corrected count increment (CCI) [180] and percent platelet recovery (PPR) [175, 179] were developed, followed by platelet count increment regression analysis to reduce result variability (Fig. 23.2) [175, 177].

Platelet refractory response is defined by two measurements in which 1-h CCI and PPR values are less than 7500 platelets/μL/m^2 and 30% and 20-h values are less than 4500 platelets/μL/m^2 and 20%, respectively [177]. Although platelet counts are higher after transfusion of apheresis platelets compared to pooled preparations, efficacy of transfusion as measured by CCI and PPR appears to be equal regardless of the method used to obtain platelets [177]. Expected count increases in an average-sized adult, as estimated by one group of investigators, are in the range of 30,000–60,000/μL for each apheresis component containing approximately 3×10^{11} platelets and 5000–10,000/μL for random donor components with an average of 7×10^{10} platelets [177]. While a "standard" platelet dose has not been defined, a reasonable estimate is the transfusion of one platelet concentrate per 10 kg of body weight and can be expected to cause a 40,000/l elevation in platelet count [177].

PLT recovery, CCI, and survival

Recovery in percentage was calculated from the formula[11]

PLT increment (after transfusion – before transfusion) [10^9/L]xblood volume[L]x100%
Transfused PLTS [10^9].

Blood volume was estimated by the formulas[12]

Women: (0.3561 x height [m]^3)+(0.03308xweight[kg])+ 0.1833=blood volume[L]

Men: (0.3669xheight [m]^3)+(0.3219xweight[kg])+ 0.6041=blood volume[L].

CCI was calculated from the formula[11]

PLT increment (after transfusion-before transfusion) [10^9/L] x body surface area[m^2]
Transfused PLTs[10^{11}].

Body surface area (BSA) was estimated by the formula[13]

BSA[m^2]=0.007184xweight[kg]$^{0.425}$xheight[cm]$^{0.725}$.

Survival was defined as flow cytometric detection of a population of transfused PLTs of more than 1% in the patients' circulation.

Fig. 23.2 Formulas for calculating platelet recovery, corrected count increment (CCI), and platelet survival post-transfusion. (Modified from Ref. [175])

Average In Vivo Life Span of Transfused Platelets

In vivo platelet circulation is determined by the rate of utilization, as well as by random and senescent mechanisms of clearance [18, 181–190]. Radioisotope-labeled platelet deposition is primarily in the spleen and liver [190], with splenic uptake occurring early, consistent with sequestration in the splenic platelet pool, whereas liver uptake occurs late and increases over time [181]. Survival studies demonstrate that platelets circulate for approximately 5–7 days and their numbers decline over time, with only about 5% remaining in circulation by day 8 [181, 191–193].

Overview of Platelet Transfusion Refractoriness

Thrombocytopenic patients can develop a clinical syndrome characterized by rapid posttransfusion platelet clearance, posing a considerable challenge if hemorrhage develops and requires treatment. While the etiology is diverse, the most common cause is alloimmunization due to frequent platelet transfusions. Platelet transfusion refractoriness is defined as an inappropriate posttransfusion rise in 1-h CCI and/or PPR (two or more consecutive CCIs of <5–10 × 10^9/l and/or two or more consecutive PPRs less than 20%, when measured at 1 h after transfusion of compatible platelet concentrates that are within 3 days of storage) [194–196]. A large prospective platelet transfusion study titled the Trial to Reduce Alloimmunization to Platelets, or the TRAP trial, was analyzed for factors implicated in platelet refractoriness. The authors found that the clinically important variables included lymphocytotoxic antibody positivity, females with at least two pregnancies, heparin administration, bleeding, palpable spleen, fever, amphotericin, and disseminated intravascular coagulation (DIC) [195–197].

Immune causes of refractoriness can be broadly described as those related to human leukocyte antigens (HLA) and those acquired against platelets, involving the human platelet antigen (HPA) system [194]. HPA-related platelet refractoriness is not only less common, less severe, and typically transient but also less preventable with leukoreduction [197]. Nonimmune causes include prior antigen exposure in multiparous females, enlarged spleen, DIC, medications [198], bleeding, fever, sepsis, veno-occlusive disease (VOD), and transfusion-associated graft-versus-host disease (TA-GVHD) [195, 199]. Component-related factors that can influence refractoriness include gamma irradiation, storage duration, and ABO compatibility [195].

Management strategies to address refractoriness start with antibody tests to differentiate between the possible immune-mediated causes (Table 23.1) [197]. If immune causes are found, HLA-matched, crossmatched, or antibody-specific platelet components are indicated. HLA-matches are categorized as A, BU, BX, C, or D, and efficacy is assessed by establishing the degree of improvement in posttransfusion CCI [200]. Crossmatching improves platelet survival [201] and, possibly, posttransfusion increments. Provision of platelets lacking the specific HLA antigens determined to cause platelet refractoriness is another method that improves posttransfusion platelet recovery and can be used if HLA-matched or crossmatched donors are not available [202]. Management for patients with nonimmune etiologies is aimed at treating the underlying causes, if possible; however, splenectomy has not proven useful when splenic enlargement is present.

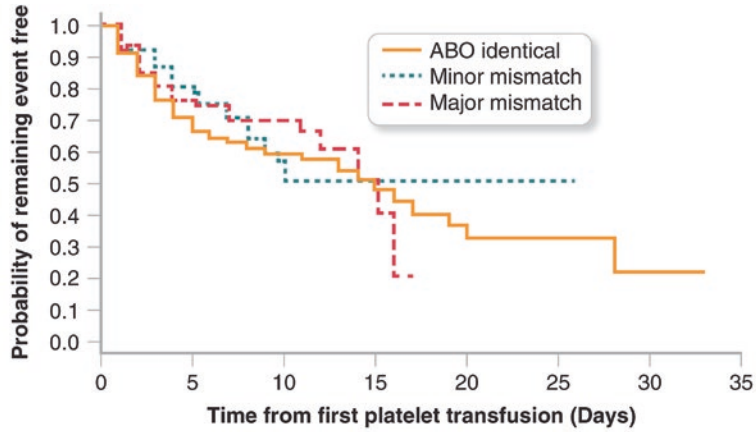
Table 23.1 Laboratory tests commonly used to detect anti-HLA or platelet-specific antibodies [197]

Lymphocytotoxicity test (LCT)
Platelet immunofluorescence test (PIFT)
Lymphocyte immunofluorescence test (LIFT)
Enzyme-linked immunosorbent assay (ELISA)
Antigen capture ELISA (ACE)
Monoclonal antibody-specific immobilization of platelet antigens (MAIPA)
Solid-phase red cell agglutination test (SPRCA)
Multiplex flow cytometric bead assays

ABO/D Compatibility for Platelet Transfusions

Accidental transfusion of ABO-incompatible red blood cells results in a severe hemolytic reaction, often leading to shock and death. The inadvisability of transfusing ABO-incompatible platelets is less clear; however, unmatched platelets are often used in clinical practice. While adverse consequences such as low posttransfusion platelet counts, acute hemolysis, fever, inflammatory changes, the development of refractoriness, and even death [203–213] can occur, particularly with major incompatibility, maintaining a strict policy of requiring crossmatched platelets can also lead to waste and platelet shortages and, in emergency cases, could also contribute to death [204]. Given the lack of clarity and the competing risks, the American Association of Blood Banks (AABB) and other regulatory bodies require blood banks and hospitals to have written policies regarding incompatible transfusion, but do not prohibit the practice [203, 204, 214]. A recent study [203] examined the data from the platelet-dose trial (PLADO) by Slichter et al. with regard to transfusion of ABO-incompatible platelets and found that, while major ABO incompatibility resulted in lower posttransfusion increments, it did not have a measurable effect on clinically significant bleeding (Fig. 23.3) [208]. Additionally, a recent study [215] in critically ill children (including organ failure, trauma, and hemorrhagic shock patients) showed no differences in posttransfusion platelet count between ABO compatible donors and those with either minor or major incompatibility. Expanding the usage of PAS would also have a positive impact on the transfusion of group O platelets to incompatible donors as anti-A and anti-B titers are further diluted [216].

Although platelets do not express Rh(D) antigen, contaminating red cells and red cell microparticles can potentially lead to alloimmunization of D− patients after D+ platelet



No. patients at risk

ABO identical	457	215	78	31	10	3	1
Minor mismatch	75	30	8	2	1	1	
Major mismatch	198	72	27	5			

Fig. 23.3 Kaplan-Meier plots of time from platelet transfusion to first >grade 2 bleeding for time from first platelet transfusion to first grade 2 or higher bleeding by ABO matching status. Time to bleeding was censored at the first date that any of the following occurred: transfusion of a platelet dose with a different ABO matching status from the patient's initial platelet transfusion or missing data on ABO matching status, missing data on whether grade 2 bleeding occurred, or end of the study. Divergence of the curves after 15 days is probably the result of the small number of patients still at risk by that time. ABO matching status was not a significant predictor of time to grade 2 bleeding ($p = 0.33$). (Modified from Ref. [208])

transfusion at rates varying from 19% to, most recently, 1.4% [217, 218]. While D− platelets and Rh immune globulin (RhIG) are recommended for D− patients of childbearing age [219, 220], the recommendations are not based on high-quality data [218, 221]. Studies in 2009 and 2015 followed D alloimmunization after D+ platelet transfusion in D− patients and reported zero or a low percentage of study subjects who developed anti-D antibodies. The authors concluded that this should be considered when determining whether to give RhIG [218, 222]. It has been suggested that ABO-incompatible platelet transfusion may actually decrease the rate of D sensitization because the residual ABO-incompatible RBCs provoke a rapid destructive response that occurs prior to the production of antibodies [223]. However, several studies [224–228] in which patients were given platelets collected by apheresis, which have the lowest RBC levels, demonstrated no alloimmunization indicating, perhaps, that a narrow window of RBC concentrations exists to produce this effect. Several reports have indicated that using D+ platelets is prudent in light of minimal risks and shortages of D− platelets [227, 229, 230], even in women of childbearing age [231]. However, for certain conditions such as chronic liver disease [232] and solid organ transplantation [233, 234], the risks may be higher.

Only a limited amount of research has been conducted on alloimmunization against other Rh antigens [235, 236], and further work is required to determine the clinical impact of those mismatches.

Practices in Leukoreduction for Platelet Units

Leukoreduction (LR) is currently the standard of care in many blood donation centers, blood banks, and medical centers due to proven and hypothesized clinical benefits (Table 23.2) [237, 238]. LR of platelet products can be accomplished in many ways, including prestorage platelet leukocyte filters, pre-transfusion platelet leukocyte filters, platelet-sparing whole blood leukoreduction, or direct leukoreduction during apheresis [239–241]. In 2013, 92.4% of all platelet units distributed in the United States were derived from single-donor platelet apheresis [242], suggesting that the majority of platelet products are leukoreduced directly during apheresis. With respect to the modulation of adverse events, leukoreduction has clear and obvious benefits associated with reduced alloimmunization, febrile nonhemolytic transfusion reactions, and CMV transmission [243–245] (Table 23.2). However, with respect to infectious agents, leukoreduction does not completely protect from TTIs, such as vCJD and *Pseudomonas*, *Staphylococcus*, and *Acinetobacter* infections [246, 247]. Other infectious agents, such as HTLVI/HTLVII and EBV, have little to no available data regarding transmission due to platelet transfusion [248, 249]. With respect to platelet refractoriness, the efficacy of leukoreduction was compared to gamma irradiation in a large, prospective, randomized trial and found to be equivalent in preventing alloantibody-mediated refractoriness [196]. In contrast, TA-GVHD, a

Table 23.2 Putative clinical benefits of leukocyte reduction

A. Proven relevant clinically:
1. Reduced frequency and severity of NHFTRs
2. Reduced risk of CMV transmission
3. Reduced risk of HLA alloimmunization and platelet refractoriness
B. Likely clinically relevant:
4. Reduced infectious risk associated with immunomodulation (TRIM)
5. Reduced organ dysfunction and mortality
6. Reduced direct risk of transfusion-transmission bacteria
C. Unproven clinically:
7. Avoidance of vCJD transmission
8. Avoidance of HTLVI/HTLVII, EBV, etc.
9. Reduced risk of GVHD
10. Reduced risk of TRALI

Putative clinical benefits of leukocyte reduction subdivided as to whether each benefit has been proven by evidence-based guidelines to be relevant clinically, likely relevant clinically, or are unproven to be clinically relevant [237]

NHFTRs nonhemolytic febrile transfusion reactions, *CMV* cytomegalovirus, *vCJD* variant Creutzfeldt–Jacob disease, *GVHD* graft-versus-host disease, *TRALI* transfusion-associated acute lung injury

clinical entity associated with high morbidity and mortality, is not preventable by leukoreduction as determined by a systematic review [250], although this data was extrapolated from older-generation filtration practices.

Pathogen reduction (PR) is an alternative method of neutralizing white blood cell (WBC) transfusion effects [251–257]. PR is effective at mitigating many newly emerging diseases [258–266], but there are still rare incidences of pathogen transmission after PR, including parvovirus B19 [267] and hepatitis E virus [268] transmission. In addition to pathogen inactivation, PR also reduces refractoriness compared to conventional platelets (no leukoreduction, irradiation, bacterial testing) [269]. While one study with a small number of patients ($n = 64$) [269] reported no differences in the incidence of TA-GVHD between PR vs conventional platelets, a review of the European experience demonstrated no cases of TA-GVHD after the transfusion of ~2 million PR (INTERCEPT®) platelets to ~300,000 patients [270]. Multiple trials of PR platelets are underway or completed: the PREPAReS randomized control trial (NCT02783313) [271], a retrospective trial of PR platelet use in children with cancer [272], a retrospective study of PR platelet use in MTP patients [273], and the EFFIPAP trial (NCT01789762) [274]. Interestingly, the PREPAReS trial demonstrated that PR does not prevent alloimmunization and that PR was independently associated with the development of de novo donor-specific antibodies to human leukocyte antigens, class I [275]. There is little data to provide insight into the incidence of TRIM in response to PR platelets. A retrospective analysis of the literature (mostly hematological/oncological patients) demonstrated that there is likely no difference in all-cause mortality when using PR platelets compared to conventional platelets but that PR platelets did lead to an increase in platelet refractoriness [276].

Adverse Effects of Platelet Transfusion

Bacterial contamination is a recognized risk of platelet transfusion due to room-temperature storage [277–280]. Pooled platelet components were ten times more likely to be contaminated compared to refrigerated RBC components [281], and recognition of increased adverse events led the FDA to reverse their decision to extend storage time beyond 5 days [282]. Within the last decade, blood banks and hospitals have implemented numerous advances in how platelets are collected, processed, and tested during storage, and these changes have resulted in reduced morbidity and mortality [279, 280, 283, 284]. Changes include improved methods of skin preparation, addition of diversion pouches to transfusion kits that reduce exposure to the potentially contaminated initial blood sample, and routine bacterial testing [285], a requirement adopted by the AABB in 2004. Adoption by many centers of single-donor apheresis platelets, which are collected in a more sterile manner, and reduced use of multiple-donor pooled platelets have also had a measurable effect on septic platelet transfusion reactions. After one center increased its use of apheresis-derived platelet concentrates from 51.7% to 99.4%, the incidence decreased by a factor of 3 [286], although a randomized controlled trial failed to document higher bacterial contamination in pooled buffy coat platelets [287]. Pooled platelet contamination risk is approximately 1 in 400; thus, the risk to a trauma patient receiving massive transfusion can surpass 1 in 100 [288]. In response to concerns about bacterial contamination, the use of apheresis platelets continues to grow ($p < 0.05$) and now accounts for 67% of platelet concentrates administered to patients according to the 2011 National Blood Collection and Utilization Survey Report [289]. Similarly, apheresis platelets are now commonly transfused in US military field hospitals, although this is primarily due to logistic considerations [75]. Despite advances in platelet collection, bacterial contamination remains a significant problem [277–279, 290, 291], resulting in related cases of bacterial sepsis and death [157, 279, 280, 292], which continue to be more common than human immunodeficiency virus (HIV), hepatitis B virus (HBV), or hepatitis C virus (HCV) transmission [293].

Due to rigorous screening and testing of blood product donors, blood product contamination with these viruses is very low, but now, attention

has turned to newly discovered threats to blood safety, with up to 5.3 previously unrecognized viruses emerging per year [294]. Examples from recent decades that have captured global attention include the variant Creutzfeldt-Jakob prion [295], West Nile virus [296], dengue viruses [297], hepatitis E [298], and Ebola viruses [299], as well as nonviral agents such as *Babesia microti* [300], *Trypanosoma cruzi* [301], and *Leishmania donovani* [302]. In response, nonspecific pathogen reduction (PR) systems were developed to address the increasing risks and costs associated with emerging pathogens and are already in use outside the United States [303]. These technologies promise to increase blood safety and reasonably preserve platelet function both in treated whole blood [22] and platelet concentrates [254]. The Intercept PR system (Cerus Corporation, Concord, CA) has gained FDA approval for treatment of platelets in 100% plasma and InterSol platelet additive solution, while a phase III clinical trial for Mirasol PR (TerumoBCT, Lakewood, CO) treatment of whole blood is currently underway.

As mentioned above, despite advances in the prevention of transfusion-related viral transmission, concerns over contamination of platelet products with infectious bacterial agents remain due to room temperature storage, and increasingly, the noninfectious sequelae of blood product transfusion are also recognized as important and commonly occurring entities. These are categorized as acute (within 24 h) and delayed (after 24 h). The most common acute transfusion reactions include febrile and allergic nonhemolytic transfusion reactions (NHTR) [304–309] and hemolytic transfusion reactions, which are the serious, life-threatening consequence of blood type misidentification. Febrile NHTR occur in response to anti-leukocyte antibodies, are typically self-limiting, can be minimized with leukoreduction, and occur most commonly in response to platelet transfusion [238, 310–312]. Allergic NHTR are distinguished by the absence of fever and presence of urticarial symptoms and can be minimized by pretreating the transfusion recipient with antihistamines [313]. Although generally mild, anaphylaxis is possible; thus, treatment often begins with halting the transfusion and monitoring the patient for development of more serious signs [314]. That said, the evidence for this strategy is not definitive [315]; thus, in the absence of a randomized controlled trial, pretreatment is relegated to physician discretion. The highest cause of death is attributed to transfusion-related acute lung injury (TRALI; Fig. 23.4) [316], defined by acute onset (≤6 h), PaO_2/FiO_2 < 300 mmHg, bilateral pulmonary infiltrates, and the absence of pulmonary edema from other causes [317]. Risk of TRALI is associated with transfusion quantity [318–320], transfusion of plasma or whole blood (particularly from female donors) [319–321], and high anti-HLA or other potentially causative antibody titers in the transfusion product [322, 323]. Platelet transfusion is not independently associated with TRALI according to several recent studies [319, 321, 322] and, according to older reports of an association with pooled platelets, may be due to the presence of plasma from sev-

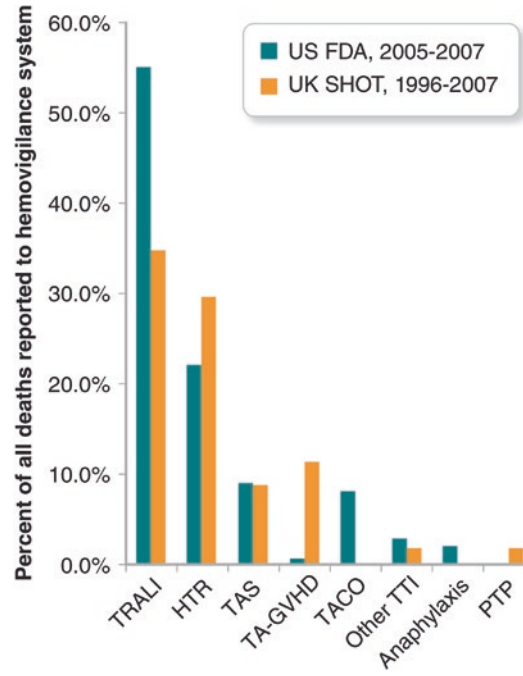


Fig. 23.4 Causes of allogeneic blood transfusion-related deaths as a percentage of all deaths reported to the SHOT (1996–2007) or the FDA (2005–2007). (Modified from Ref. [316])

eral donors [324], particularly if leukocytes and/or high antibody titers are present. The etiology is predominantly due to a combination of activated pulmonary endothelium and recruitment of polymorphonuclear leukocytes (PMNs) due to trauma or critical illness, followed by the transfusion of leukocyte-containing blood products [325]. Up to 40% of patients with TRALI have only been exposed to leukoreduced blood products, and thus, other etiologies occur that have yet to be fully described.

Late complications of transfusions include posttransfusion purpura (PTP) and transfusion-associated GVHD. PTP is a rare, but serious, entity typically due to anti-HPA-1a (an antibody against a platelet-specific antigen) [326], is most common in parous women who have been sensitized to platelets during pregnancy, and is treated with high dose intravenous immunoglobulin. TA-GVHD is characterized by rash, fever, pancytopenia, and liver dysfunction [327]. Multiple etiologies for TA-GVHD, which is associated with greater than 90% mortality, have been described, involving immunocompromised and immunocompetent patients. Risk factors associated with this life-threatening syndrome include immunocompromise, transfusion of HLA homozygous blood into heterozygous patients, intrauterine transfusion, advanced recipient age, and short RBC (<11–14 days) storage times, thus greater probability of containing active lymphocytes [327, 328]. When immunocompetent patients develop TA-GVHD, reactions are more likely to involve an incomplete mismatch that allows the persistence of donor lymphocytes [328, 329]. Common symptoms are fever, erythema, and leukocytopenia [328]. Therapeutic intervention is primarily focused on prevention, which is primarily accomplished by gamma irradiation of transfused products to inactivate T lymphocytes, due to the difficulty of reversing the disorder once bone marrow is compromised and to the high mortality associated with this syndrome [329, 330]. Incidence markedly declined once widespread irradiation of transfused components was widely adopted for patients at risk [328].

Indications and Dosing for Platelet Transfusion in Injured Patients

Although physicians agree that platelet transfusion is lifesaving for profoundly thrombocytopenic bleeding trauma patients, there is no high-quality data to guide resuscitation, nor consensus regarding the timing of transfusion for nonthrombocytopenic patients with massive hemorrhage. Evidence-based guidelines are needed to establish transfusion thresholds and dosing strategies. Research gaps are due to the ethical issues which proscribe studies comparing platelet unit transfusion to placebo, as well as the technically challenging nature of prospective platelet dosing studies. Platelet functional deficits are recognized, but the degree of dysfunction is highly variable, and in vivo methods of measurement are not validated in trauma patients. As mentioned above, commonly accepted indications for platelet unit transfusions include the treatment of active bleeding with thrombocytopenia (<50,000/μL or < 100,000 in the presence of disseminated intravascular coagulation (DIC) or central nervous system bleeding), in preparation for an invasive procedure in a thrombocytopenic patient and for prevention of spontaneous bleeding in high-risk patients with thrombocytopenia [331]. Although there are theoretical benefits, these thresholds are not based on evidence of efficacy in improving outcomes or reducing blood loss or blood product utilization. Until recently, actively bleeding trauma patients received platelet transfusions only after thrombocytopenia was documented, but this strategy is currently being challenged by physicians who have reason to believe that severe traumatic life-threatening bleeding could rapidly lead to death in this vulnerable population. As noted above, recent studies suggest that platelet function can be compromised in response to trauma despite platelet counts above classic target thresholds for transfusion [332] and that early empiric use of platelets is associated with improved outcomes [78, 82, 333, 334]. For all these reasons, empiric platelet transfusions are becoming standard practice in addressing traumatic hemorrhage [80, 335].

As previously discussed, military clinicians treating casualties of the recent conflicts in Iraq and Afghanistan were faced with a degree of injury and number of cases rarely if ever equaled in civilian trauma centers. In response to their experience with mass casualty activations, they noted that outcomes improved when patients with traumatic hemorrhage received platelet replacement during the initial resuscitation. Perkins et al. reported in a cohort of 464 patients with combat-related trauma that the use of platelets was independently associated with improved 30-day survival [78]. Brasel et al. published that increased platelet-to-RBC-unit ratios were associated with survival in patients with and without severe traumatic brain injury in a civilian cohort of patients [333]. An increased ratio of platelet to RBC units transfused was associated with increased survival in a 10-year review of patients with combat-related injuries by Pidcoke et al. and in civilian cohorts by Holcomb et al. [82, 334]. One small prospective RCT of 33 massively bleeding trauma patients compared transfusion of 6 units of random donor unit platelets to 2 units of fresh frozen plasma (FFP) administered with every 12 units of modified whole blood [336]. In this study, there was no difference in the incidence of microvascular bleeding between the two study groups, but results from the PROPPR trial demonstrated that hemostasis was achieved in more patients treated with the higher ratio of platelets and fewer expired due to exsanguination [80].

The use of platelet units for patients without life-threatening bleeding is not well studied; however, a retrospective analysis of 1788 transfused trauma patients treated with resuscitations that did not meet massive transfusion criteria (<10 units of RBCs in 24 h) reported that higher platelet (or plasma)-to-RBC-unit ratios were not associated with increased survival but rather with increased morbidity represented by reduced intensive care unit (ICU)-free and mechanical ventilator-free days [337]. The degree to which these observations are attributable to specific platelet effects versus overall transfusion volume is difficult to ascertain and suggest the need for further exploration in randomized clinical trials.

The optimal dose of platelets to treat patients with life-threatening traumatic injury is unknown. It is unlikely that a single dose can be identified since the degree of thrombocytopenia and reduced platelet function are highly variable due to the heterogeneity of traumatic injuries and wide variation in time from injury to the presentation at a treatment facility with platelet units available. Platelet transfusion is based upon clinical response and typically given at a ratio of 1:1 to 1:2 platelets to RBC units. A recent survey of 132 US trauma centers indicated that 79% of centers target a platelet-to-RBC-unit ratio greater than or equal to 1:2 for patients that require massive transfusion protocol activation [335].

In opposition to those who advocate empiric platelet transfusion, a growing body of literature supports the so-called "goal-directed" hemostatic resuscitation based upon measures of platelet function, which can be measured by viscoelastic tests or platelet aggregometry [338]. Multiple platelet transfusion algorithms in patients with traumatic injury have been published. Some are applicable to patients with traumatic injury, but thresholds and therapeutic targets have not been formally validated [339]. While the use of functional platelet measures to drive goal-directed hemostatic resuscitation based on therapeutic targets appears more rational than merely targeting an arbitrary number of platelets, particularly in the light of recent data describing early platelet inhibition after severe traumatic injury, the evidence supporting this strategy remains inconclusive.

Whole Blood as a Source of Platelets

Whole blood, a product licensed by the FDA for up to 21 days of storage at 4 °C in citrate phosphate dextrose (CPD) or 35 days in citrate phosphate dextrose-adenine 1 (CPDA-1), is another, potentially more optimal, source of platelets for patients with traumatic hemorrhagic shock. A whole blood unit is less dilute compared to a comparable unit of reconstituted whole blood made from individual components of RBCs,

plasma, and platelets, and it has the advantage of being simple to administer and rapidly available while allowing for the simultaneous treatment of both coagulopathy and shock [340].

Until recently, the clinical availability of whole blood had been limited since the 1970s due to three main concerns. Whole blood had to be ABO-specific, and maintaining sufficient inventory to ensure a ready supply of every ABO type would result in significant cost and waste. The second reason stemmed from the misperception that whole blood cannot be leukocyte-reduced, a process that typically removed platelets with the leukocytes and thus cannot be considered a platelet-containing product. The FDA has now approved a WB leukoreduction filter that is platelet-sparing, paving the way for leukoreduced WB as a platelet-containing product. A final concern was that the platelets in whole blood stored at 4 °C are not functional or viable, once again calling into question whether WB is truly a platelet-containing product. As reviewed earlier in this chapter, this misconception stemmed from the spurious assumption that spherical platelets are nonfunctional. Cold-stored platelets, despite irreversible shape change, aggregate better, are able to form a thrombus, and are associated with stronger clot formation when compared to the current standard of RT-stored platelet concentrates [21, 22, 55]. Due to 4 °C storage, platelets in whole blood are more hemostatically active compared to standard-of-care (22 °C) platelet components. However, while ABO-specific fresh whole blood has been used at some children's hospitals for over a decade [341], it has not been routinely available for adults until recently in the form of low-titer group O whole blood.

With respect to the treatment of trauma and hemorrhagic shock, much evidence has been gathered in since 2015 to show that whole blood may, in fact, be the best choice for resuscitation in terms of safety, efficacy, and ease of use, especially in the prehospital setting [342, 343]. The AABB standards for ABO specificity were modified in 2018 to allow for whole blood usage with only RBC compatibility required [344], and several in vitro studies [345–348] have shown that stored whole blood platelets retain aggregation, thrombus adhesion, clot formation, and thrombin generation functions despite the decline in platelet count and coagulation factor performance over storage duration. Additionally, several clinical efforts have been completed or are underway demonstrating the utility of whole blood [349–352]. Because of these data, whole blood is now being used in at least 70 trauma centers in the United States.

Due to the concern of immunomodulation with non-leukocyte-reduced products and regulations in many countries that require it, the use of leukoreduction with a platelet sparing filter is performed in some US trauma centers. To date, there have been no clinical trials assessing the safety and efficacy of leukoreduced whole blood compared to non-leukoreduced whole blood. However, there have been a handful of studies assessing the hemostatic efficacy of leukoreduced whole blood. The consensus of these publications is that the platelet sparing filter used during the leukoreduction process causes an immediate decrease in platelet aggregation and a more gradual decline in hemostatic function (assessed by viscoelastic measurements) [353–356]. Leukoreduced whole blood was also found to have preserved fibrin polymerization [357], as well as decreased accumulation of lysophosphatidylserine and increases sphingosine-1-phosphate [358].

Type-Specific Versus O-Negative and O-Positive Low-Titer Whole Blood

Transfusion of low-titer group O whole blood (LTO-WB) is an alternative solution to type-specific whole blood (TSWB), but despite being the standard of care for treating hemorrhagic shock up to and during the Vietnam War [359], it was not permitted by AABB standards prior to 2018 [360]. The rationale for this prohibition was difficult to understand since hundreds of thousands of units of type O whole blood, with low anti-A and anti-B titers to reduce the risk of plasma incompatible ABO reactions, were used

during both World Wars, the Korean War, and the war in Vietnam [359, 361, 362]. The threshold for low-titer set by the US military in the Korean War was <1:256 [363]. A report by Nessen et al. indicated that, at US military forward surgical bases, the use of type O whole blood was associated with improved outcomes when compared to RBCs and plasma alone [364]. In addition to decades of use with few reports of adverse complications, there are multiple reasons to suppose that LTO-WB is safer than TSWB. While a small risk of severe hemolytic reaction to LTO-WB does exist, it is similar to that of transfusing type O RBCs, which are currently available for emergency release at major trauma centers. It is the plasma contained in the blood that causes the greatest concern for ABO incompatibility, but the associated hemolytic reaction is typically mild to moderate, and the incidence, only 1:120,000 transfusions in the United Kingdom's Serious Hazards of Transfusion (UK SHOT) database, is low [365]. In contrast to this nonfatal risk, type-specific WB transfusion carries a higher risk (1:80,000) of severe hemolytic reaction, mainly due to human error [365].

Given the prevalence of type O (and especially O+) donors, LTO-WB and LTO + WB are viable alternatives to type-specific WB, but to date, these continued to be largely unavailable. Implementation of LTOWB requires a method for determining titers, but multiple assays are available with a lack of evidence-based standards. In addition, a commonly accepted, data-driven threshold that defines "low titer" anti-A and anti-B immunoglobulin M (IgM) and IgG is needed to replace the arbitrary values used in previous military conflicts. There has been no standardization of measurement methodologies or threshold definitions [366, 367] although recent efforts have attempted to determine best practices [368] and, therefore, the AABB has allowed each institution to make its own determination of the definition of "low titer," so long as such a determination is made. However, repeat testing in identified "low titer" donors has shown that their titer levels do not increase over time [369]. In the report by Nessen et al. described above, the authors also found that use of untitered type O whole blood was not associated with increased adverse events [364], but outside of emergent cases or very austere environments, few would advocate this strategy given the small sample size and the relatively low cost of establishing titers.

In considering the advantages and disadvantages of the whole blood products discussed, it is clear that type-specific WB, originally chosen by regulatory bodies for its apparent safety, in actuality, carries the highest risk because of the incidence of human error and is the least cost-effective of the three. The challenges associated with maintaining a large inventory of every blood type, and the inevitable waste, relegate this option to a minor role at best for the treatment of hemorrhagic shock. Similarly, continued use of blood components for severely bleeding trauma patients should undergo reevaluation due to the possibly unnecessary financial burden of fractionation, the poor quality of platelet function, the added encumbrance of bacterial testing required for platelets stored at 22 °C, and the cost of frozen plasma wasted due to packaging fractures during the thawing process. In contrast, the cost of whole blood expiring before it can be used to treat hemorrhagic shock may compare favorably, even after factoring in the cost of establishing titers.

According to a 2015 survey of 132 trauma centers, prehospital blood products were only used for resuscitation by 34% of first responders [335]. A smaller minority were carrying both RBCs and plasma and very few, if any, carried platelets, although multiple studies demonstrate that they are important for hemostasis. More recently, LTOWB usage has increased in the prehospital realm, with documented illustrations of the safety as well as the benefits of this product [370–372]. The use of blood products to resuscitate patients in the prehospital phase has the potential to reduce the large risk of death from hemorrhagic shock prior to hospital admission. Military data indicates that 90% of potentially survivable deaths occur from hemorrhagic shock [373]. Improved control of bleeding and early blood product use are required to reduce this toll. The logistical constraints of prehospital transporting RBCs, plasma, and platelets are considerable. In addition to the extra weight and

complexity of transfusing products from multiple bags, intravenous or intraosseous catheters have a limited number of access ports. The use of whole blood at 4 °C stored for less than 10–14 days dramatically reduces the logistical burden compared to the current approach, which too often provides the oldest component products in the inventory. Storage lesions can include fragile RBCs prone to hemolysis, thawed plasma with attenuated thrombin generation potential, and RT platelets with the deficits more fully described above. LTOWB, in addition to being safer, lighter, and logistically supportable, may also be more efficacious in providing hemostatic function. In summary, for patients with traumatic injury and hemorrhagic shock, the current component products may not be optimal, but until further research leads to changes in regulations and guidelines, preemptive transfusion of RT-stored platelets in massively bleeding patients, in advance of thrombocytopenia, is associated with better outcomes. Refrigerated platelets may improve safety, and stored whole blood is an underutilized potential source of platelets, plasma, and RBCs that may be more logistically feasible both in the hospital and, recognizing the increased use of blood products during transport, in the prehospital setting. Due to safety, logistical, and cost considerations, type-specific WB is not a feasible option for widespread deployment; thus, adoption of LTOWB (and particularly, LTO+WB) as the primary resuscitative fluid for massive hemorrhage would require a change in AABB guidelines beyond what has already been granted.

Future Directions in Platelet Transfusion Research and Areas of Opportunity for Investigators

Platelets came to occupy their functional niche through complex and poorly understood evolutionary pressures. Unlike invertebrates, birds, and fish, only mammals possess anucleate platelets derived from polypoid megakaryocytes that are confined to the bone marrow [272, 273, 374, 375]. Megakaryocytes and platelets evolved from ancestral diploid cells that performed both phagocytic and hemostatic functions [376]. Most likely due to enhanced activation signaling through ADP receptors and increased expression of the fibrinogen receptor, glycoprotein IIb/IIIa (GPIIb/IIIa), mammalian platelets are capable of forming occlusive, shear-resistant thrombi with an ability to change shape that is unhindered by a nucleus. This enhanced hemostatic capacity may have provided mammals with an evolutionary advantage by improving survival after trauma. On the other hand, these evolutionary changes also set the stage for the mammalian scourge of thrombotic cardiovascular disease [374]. The role of platelets in the response to injury is complex and includes hemostatic, immune, and trophic aspects [377–379]. The megakaryocyte response to injury is not confined to increasing platelet production, but details regarding additional functions remain relatively obscure. Interactions between these cells and the endothelium, other immune effector cells including the hepatosplenic and other components of the reticuloendothelial system, and stem cells are potentially fruitful areas of research. In addition to probing the role of platelets at the nexus of the hemostatic and immune systems, platelet product development, whether addressing challenges in platelet preservation, development of synthetic hemostatic platelet substitutes, or even exploitation of platelets as drug delivery vehicles, remains an underexplored scientific frontier. As to clinical applications that require further elucidation, much work remains to be done to optimize platelet products and transfusion strategies for both medical and surgical patients, but several themes are being clarified. In treating severe, acute hemorrhage, platelet consumption and loss must be addressed early and aggressively to achieve the best possible outcome. Functional platelet counts matter, and an "adequate" platelet count does not equate with adequate platelet function. The dogma that platelets should be stored at RT and transfused prophylactically in the setting of hypoproliferative thrombocytopenia has been challenged, and the assumption that platelet counts above 50,000 are adequate for trauma patients is refuted by the PROPPR study. In short, the field of platelet transfusion is at a crossroads,

and both regulators and scientists are called to critically reexamine current standards and recognize the importance of updating our understanding of the platelet.

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24 Whole Blood for the Resuscitation of Massively Bleeding Civilian Patients

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The Rationale for Intervening Early in the Resuscitation with Blood Products

Traumatic hemorrhage is a leading cause of death and disability, especially in younger adults [1], and traumatic hemorrhagic shock in adults has a mortality approaching 20% at 24 hours post-injury [2]. A massively bleeding patient should be resuscitated with fluids that closely resemble what they are bleeding in order to maintain tissue oxygenation and promote hemostasis. However, for many years, resuscitation protocols focused on the early and aggressive use of crystalloids, such as normal saline, because they were inexpensive, easily transported at room temperature in resilient plastic bags, and did not carry with them the risks of transfusing human blood products [3]. It was thought that if the patient's hemodynamics could be maintained using crystalloids, then the large physiologic reserve of hemoglobin in red blood cells (RBC) and clotting factors in plasma and the extravascular space would reach their respective tissue destinations and perform their functions [4]. Guided by this dogma, liters of crystalloid fluids were routinely transfused to massively bleeding patients, as neither the acidic nature of normal saline nor the potentially beneficial effects of permissive hypotension had yet been appreciated [5–7].

Recent studies have highlighted the disadvantages of overzealous crystalloid resuscitation in trauma compared to resuscitation strategies using early intervention with blood products [8–12]. Perhaps, the most influential study supporting this notion was that of Bickell et al. [13]. In this study, hypotensive patients with gunshot or stab wounds to the torso were randomized to receive crystalloid therapy before surgery including during transport to the hospital (early) or to only receive fluids during their surgical procedure (delayed). The randomization was quite effec-

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tive: the patients in the early fluid intervention group received an average of 870 ± 667 ml of crystalloid fluid in the prehospital phase of their resuscitation compared to an average of 92 ± 309 ml in the delayed resuscitation group ($p < 0.001$). Similarly, the volume of crystalloid administered after arrival at the trauma center and before the surgery was also significantly higher in the early intervention group compared to the delayed intervention group (1608 ± 1201 ml vs. 283 ± 722 ml, respectively, $p < 0.001$). Not surprising by today's standards, there was an 8% reduction in mortality in the delayed group that only received fluids during their surgical intervention compared to the early fluid resuscitation group, and the former group also had a significantly shorter average hospital length of stay, without an increase in postoperative complications [13].

Building on these data and supporting the early intervention with blood products instead of crystalloid in traumatically injured patients, a study of 502 military combat casualties demonstrated that the provision of primarily RBCs within approximately 30 minutes of injury improved both 24-hour and 30-day survival compared to patients who did not receive any blood products or who received them later in the resuscitation [14]. In the multicenter Prehospital Air Medical Plasma (PAMPER) trial, civilian trauma patients, whose median helicopter transport times to the hospital were approximately 40 minutes, were randomized to receive two units of plasma in addition to the standard of care treatment while *en route* to the hospital. This study found that 30-day mortality was improved compared to patients who received the prehospital standard of care, which in many cases was crystalloid fluid only [15]. In a secondary analysis of this trial, the greatest survival benefit was demonstrated among those who received RBCs and plasma compared to those who received plasma alone. In fact, receipt of any blood product during prehospital resuscitation yielded a significantly improved 30-day survival rate compared to patients who received crystalloids alone. Additionally, compared to those who received any blood products during the helicopter transport, each liter of crystalloid that was administered was associated with a 65% increase in 30-day mortality [16]. Furthermore, a secondary analysis of the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial that compared outcomes of trauma patients who were resuscitated with two different blood products ratios found a 5% increase in mortality for every minute that blood products were not provided to a trauma patient after the massive transfusion protocol (MTP) had been activated [17]. In addition, a recent retrospective analysis of nearly 57,000 US military casualties in Iraq and Afghanistan revealed that the increasingly rapid availability of blood transfusions, in conjunction with the increased use of tourniquets and faster medical evacuation of the casualties resulted in a 44.2% reduction in mortality [18]. These findings underscore the importance of having blood products, not crystalloids, available early in the resuscitation of massively bleeding trauma patients. Improving the prehospital care of massively bleeding patients will save lives: more than half of civilian preventable prehospital deaths are due to hemorrhage [19], and approximately 85% of the 30,000 preventable deaths that occur every year in the United States happen before the patient arrives at the hospital [20, 21].

Reenter Whole Blood: A Novel Rediscovery of an Old Idea

Not all blood products are created equally. While it might seem reasonable to assume that transfusing a unit of RBCs, plasma, and whole blood-derived platelets (PLT) would be functionally and volumetrically equivalent to transfusing a unit of whole blood, there are major differences between these products [22]. Table 24.1 demonstrates the quantities of anticoagulant-preservative and additive solutions that are added to different blood components. Whole blood, that is, blood that is not manufactured into its component parts, is only diluted with the 70 mL of citrate-phosphate-dextrose (CPD) solution required to preserve RBC viability for 21 days of refrigerated storage. The 110 mL of additive solution (AS) that is

Table 24.1 The quantity of preservatives and anticoagulants in various blood products

Blood product	Volume of CPD (mL)	Volume of AS (mL)	Total volume of preservatives and anticoagulants (mL)
Plasma	48	0	48
Red blood cell	8	110	118
Apheresis platelet	35	0	35
Whole blood-derived platelet	14	0	14
Whole blood	70	0	70

Derived from Ref. [22]
CPD citrate phosphate dextrose, *AS* additive solution

added to each RBC unit in order to extend the shelf life of the unit to a maximum of 42 days (depending on the jurisdiction) adds a significant volume of fluid that neither promotes hemostasis nor carries oxygen. Thus, if a massive transfusion of 10 units each of RBCs, plasma, and whole blood-derived PLTs was administered, an estimated 1800 mL of CPD and AS would be infused to the recipient along with the blood components themselves. By contrast, a resuscitation performed using 10 units of whole blood would infuse only 700 mL of CPD and additive solution. The extra citrate-containing, non-oxygen-carrying anticoagulant and RBC nourishment fluid that is administered when conventional components are transfused can be significant: a computer simulation of a 20-unit massive transfusion event demonstrated that when whole blood was used starting in the prehospital phase of the resuscitation and continued until the bleeding was controlled in the operating room, the patient's total extracellular fluid compartment was nearly 1 L smaller than if conventional components had been utilized [22]. Avoiding increased extracellular fluid is essential in critically ill patients since it increases the risk of ARDS and organ failure related to anasarca [23].

Whole blood units also contain PLTs if prepared using a platelet-sparing leukoreduction (LR) filter or if the WB is not leukoreduced at all. There have been concerns about the detrimental effects of cold storage on the PLTs in whole blood, since cold-stored PLTs are cleared from circulation much more quickly than conventional room-temperature-stored PLTs due to changes in the sialyation of certain membrane receptors [24]. However, these cold-stored PLTs demonstrate superior in vitro hemostatic properties compared to room-temperature-stored PLTs [25–27], suggesting that they might be primed to promote coagulation once transfused. Unlike hematology/oncology patients who require prolonged hemostasis over several days to prevent spontaneous bleeding, a massively bleeding patient needs short-term hemostatic support until the bleeding can be permanently controlled during surgery and the few hours that the cold-stored PLTs from whole blood circulate should be enough to provide hemostasis for a patient experiencing an acute massive bleed (see below) [28]. In fact, several earlier studies have hinted at the in vivo superiority of cold-stored PLTs compared to room-temperature-stored PLTs [29, 30], but this assertion awaits definitive clinical confirmation in the trauma or massively bleeding patient populations. Furthermore, the aforementioned computer simulation of a massive transfusion event indicates that the exclusive use of whole blood during the resuscitation facilitates a higher and more consistent PLT count in the recipient, avoiding the peaks and troughs that are associated with transfusing PLTs in a goal-directed manner, that is, based on conventional laboratory testing such as a PLT concentration determination [22].

Now that the lifesaving nature of prehospital transfusions is becoming better appreciated, transporting whole blood in place of RBCs in emergency vehicles should make early balanced blood product support easier. Thus, the use of WB will greatly simplify the logistics of the resuscitation by providing balanced resuscitation in one bag instead of up to three bags that all have to be separately procured from the blood bank and stored under two different conditions (i.e., RBCs and liquid plasma between 1 and 6 °C and PLTs at room temperature). This is especially important in the prehospital setting where storage space in helicopters and ambulances is limited,

Table 24.2 Advantages of using group O whole blood over conventional components in massively bleeding patients

Simplifies the logistics of the resuscitation by providing a balanced resuscitation fluid in one bag instead of three
More concentrated product compared to reconstituting whole blood with conventional components
Provides cold-stored platelets that have improved in vitro and perhaps in vivo hemostatic function compared to room-temperature-stored platelets in this patient population
Provides for a longer shelf life for stored platelets compared to room-temperature storage
Provides for the availability of platelets where they might otherwise not have been available
Reduces the bacterial contamination rate of a platelet-containing product
Perhaps reduces the incidence of ABO mis-transfusion during the resuscitation

and often, intravenous access to the patient is limited, thereby reducing the speed by which the patient can be separately resuscitated with individual RBC, plasma, and PLT units.

Table 24.2 lists many of the advantages of transfusing whole blood compared to conventional components.

Whole Blood Collection, Manufacturing, and Storage Practices

Previously, the AABB (formerly known as the American Association of Blood Banks), the organization that establishes standards with which accredited blood banks and hospital transfusion services must comply, required that WB had to be ABO-*identical* with the recipient. This standard greatly limited the use of WB in massively bleeding patients, as early in the resuscitation the ABO group of many such recipients is not known, yet their need for blood products can be high [31]. However, starting with the 31st edition of their standards in 2018, WB that is ABO-*compatible* with the recipient was permitted [32]. Effectively, a unit of WB must be group O in order to be safely transfused to any recipient whose ABO group might not be known at the time of the transfusion [33]. Using group O WB will avoid causing an acute hemolytic reaction mediated by the naturally occurring anti-A and/or anti-B that are present in all non-group AB recipients. However, group O WB necessarily contains anti-A and anti-B in its approximately 250 mL plasma component, which will be incompatible with all non-group O recipients. Therefore, another AABB requirement for using WB when the recipient's ABO group is not known (or is known to be non-group O) involves mitigating the risk of hemolysis caused by the anti-A and anti-B that are present in all group O WB units. Hemolysis risk mitigation comes in two parts: ensuring the transfusing hospital has a policy on what antibody titer constitutes a low titer and determining the number of low-titer, group O whole blood (LTOWB) units that each patient can receive.

Antibody Titers

Each unit of WB must undergo antibody titering and be shown to be below the hospital's selected titer threshold before it can be issued to a patient whose ABO group might not be known. A group O WB unit that has anti-A and anti-B titers below the hospital's threshold is known as an LTOWB unit. Determining each hospital's titer threshold is based on the hospital's tolerance of risk and the blood center's ability to supply these units. It is likely that any titer <256 will be safe in these bleeding patients based on the experience of transfusing ABO minor-incompatible PLTs and of using LTOWB itself [34]. Two recent surveys of primarily United States-based hospitals that use LTOWB found that the most common definition of low titer was <200, as this is the titer threshold used by a large supplier of LTOWB units, although there was a range from <50 to <256 [35, 36]. Interestingly, one respondent to the survey indicated that they had two titer threshold criteria; at this center, a unit of WB is considered to have low antibody titers if the IgM titer is <256 and if the IgG titer is <500.

The serological safety of transfusing ABO minor-incompatible plasma was recently demonstrated in the safety of the use of group A plasma

in trauma (STAT) study [37]. This study found no differences in a variety of mortality outcomes and also the length of hospital stay between group A recipients of group A plasma during their trauma resuscitation compared with group B and AB recipients of group A plasma. Furthermore, there is an extensive history of transfusing ABO minor-incompatible PLTs with few reports of hemolysis [38], and low-titer group A plasma was safely transfused in the PAMPER study [15]. Several reports of transfusing LTOWB to civilian trauma casualties have also not found evidence of hemolysis among the non-group O recipients, who are potentially at risk of hemolysis from the anti-A and anti-B in an LTOWB unit, compared to the group O recipients whose RBCs are not at risk of hemolysis from receipt of LTOWB [39–41]. It should be noted from these and other studies [42] that the biochemical markers of hemolysis when measured in traumatically injured patients receiving massive transfusion tend to be perturbed in the same way as a patient who is experiencing immune hemolysis such as during an acute hemolytic transfusion due to the administration of an ABO-incompatible RBC unit. In both cases, the haptoglobin concentration decreases [43, 44], and the lactate dehydrogenase (LDH) and total bilirubin concentrations increase. In trauma patients, these changes are due to the mechanical lysis of RBCs caused by the trauma itself, the liberation of LDH from tissues other than RBCs, as well as potentially the rapid infusion of RBC-containing blood products, whereas in a patient undergoing hemolysis the changes in these parameters would be due solely to the intravascular destruction of the RBCs.

How frequently donors should be tested to ensure that they have low titers of anti-A and anti-B is also an unanswered question. It is known that the titers of anti-A and anti-B can change with diet [45], and sometimes following the receipt of some [46], but not all [47], vaccines. However, a recent multicenter study did not find any seasonal periodicity in the rate of detecting high-titer whole blood and apheresis PLT donors over a 24-month period [48]. Two Danish studies that followed the anti-A and/or anti-B titers of healthy blood donors and laboratory staff [49], as well as patients on chronic hemodialysis [50], every 3 months for a year also did not find substantial variation in the titers between donors over time. This would suggest that most donors' titers remain relatively constant over time. However, a study of nearly 2000 elite group O US soldiers who had their anti-A and anti-B titers tested after completion of their training or prior to deployment to determine their eligibility to serve as LTOWB donors during combat missions found a statistically significant increase in the number of high-titer ($\geq$256) donors when the titering was performed in the autumn compared to the winter [51]. Curiously, this study also found that the probability of a solider having low antibody titers increased with the number of times that they were tested; in another study of military blood donors, almost 20% of high-titer donors were found to have low titers on a subsequent screening test [52]. These interesting military data warrant further investigation. Thus, each blood center will have to determine how frequently to titer their donors based on these data and on emerging data that demonstrates an association between high titer frequency and donor age and ethnicity [53].

For comparison with the civilian practice, 75th Ranger Regiment, a US Army Special Operations Forces unit, has recently implemented a new local blood collection protocol where soldiers are registered as blood donors prior to a combat deployment [54]. All group O donors that are accepted as "universal donors" are prescreened for both anti-A and anti-B, and individuals with titers <128 are identified as "universal donors" and are used for local blood collection and "buddy transfusions" with LTOWB under field conditions [52, 55, 56].

As far as which laboratory method should be employed for titering the WB units, a recent study revealed that performing a 1-dilution titer, that is, diluting the donor plasma to the titer threshold with saline and testing the diluted plasma without an incubation period using the saline tube, column agglutination (gel), or automated microplate techniques produced results with approximately equal accuracy, and similar

positive and negative predictive values compared to a reference saline tube technique that included a 1-hour room-temperature incubation [57]. Laboratories should consider the number of samples they will be testing, the required turnaround time, volume of samples to be tested, the sophistication of their technologists, and the nature of the equipment available for performing the titers when selecting their titer method.

Hospital Policy Specifying the Maximum Quantity of LTOWB Units Per Patient

The new AABB WB Standard also requires each hospital to determine how many units of LTOWB each patient can receive per transfusion episode to mitigate the risk of hemolysis. Note that a policy that does not specify a maximum number of LTOWB units that a patient can receive would be compliant with the standard, as long as the policy was clear that the hospital does not desire to limit the number of LTOWB units that each patient can receive. For example, a recent case report detailed the transfusion of 38 units of LTOWB to a traumatically injured recipient at a hospital where their policy does not specify the maximum number of LTOWB units per patient, and their inventory consists of up to 40 units of LTOWB [58].

Other Safety Considerations for LTOWB

As an entire unit of plasma is transfused with each LTOWB unit, any transfusion-related acute lung injury (TRALI) risk mitigation strategies that a blood center employs for conventional plasma or apheresis PLT units should also be employed when selecting LTOWB donors. Typically, these strategies involve collecting LTOWB units from females without a pregnancy history or those who have been tested and found not to have become HLA-sensitized or from male donors who naturally have a low risk of HLA alloimmunization because the main etiology of HLA sensitization is pregnancy [59, 60]. Other considerations for selecting LTOWB donors include whether the donor should be D+ or D−; this is a controversial issue, and once again, the decision requires a balance between the transfusing center's tolerance of the risk of D alloimmunization among D− recipient of D+ LTOWB versus the blood center's ability to supply D− LTOWB units that also meet all of the other qualifying criteria [33]. Some centers, such as the city of San Antonio, Texas, United States, and some of its surrounding areas [61], provide exclusively D+ LTOWB to all eligible trauma patients regardless of their gender and age (as long as the recipient is ≥5 years old) because they historically have had very few D− females of childbearing age that have required a massive transfusion in trauma; this center demonstrated that of 124 massive transfusion protocol activations over a 30-month period, there was only one woman of childbearing age who underwent pretransfusion testing and was found to be D− [62]. Other centers, such as the University of Pittsburgh Medical Center (UPMC) in Pittsburgh, Pennsylvania, United States, only provide D+ LTOWB to male patients of any age or females who are ≥50 years old; females who are <50 years of age receive conventional components including D− RBCs and PLTs during their trauma resuscitation until their D type is determined [63]. The exception to this policy is at the Children's Hospital of Pittsburgh of UPMC in Pittsburgh where D− LTOWB is provided to all traumatically injured boys and girls who are ≥1 year old and who weigh ≥10 kg (children who are <1 year old and <10 kg are resuscitated using conventional components at this center). In San Antonio, Texas, male and female patients older than 5 years of age can receive D+ LTOWB in the prehospital setting if they are traumatically injured, and LTOWB administration can continue once they arrive at the hospital, typically at in aliquots of 10–20 ml/kg although there is no formal limit on the quantity of LTOWB that can be administered. If traumatically injured pediatric patients do not receive LTOWB in the prehospital setting, they must be at least 10 years of age to receive it in the hospital, and there is similarly no limit on the quantity

of LTOWB that could be administered. Only D+ LTOWB is provided in San Antonio to both eligible boys and girls (D. Jenkins, July 2019, "personal communication"). Further details of other American and international LTOWB programs can be found elsewhere [35, 36].

Ideally, all females of childbearing potential whose D type is unknown should receive D− cellular blood products until they are shown to be D+. Unfortunately, only approximately 8% of the US donor population is O− [64]. Thus, finding qualified donors who have low antibody titers, are not HLA alloimmunized, and are blood group O− is very difficult. For example, at a large blood collector in the United States where 8% of the donors are O−, 48% are male (only males are used at this blood collector as a TRALI risk mitigation step for LTOWB), and 80% have low titers of anti-A and anti-B, only approximately 3% of all donors would qualify to donate *O*-LTOWB. This fraction increases to approximately 15% of all donors who would be eligible to donate *O*+ LTOWB. As alluded to above, the calculus on whether to provide D+ LTOWB (or D+ uncrossmatched RBCs for that matter) for patients of unknown D type who require urgent transfusion requires balancing the following considerations: it is known that the rate of D alloimmunization among hospitalized D− recipients of at least one unit of D+ RBCs is approximately 22% [65–67]. Anti-D can cause very severe hemolytic disease of the fetus, and newborn (HDFN) should a female of childbearing potential become alloimmunized. It is also known that the probability of a Caucasian fetus carrying the D antigen is approximately 85% and that the rate of experiencing the most severe outcomes of HDFN, such as requiring intrauterine transfusions or fetal demise, is approximately 30% [68]. Thus, the rate of becoming D alloimmunized and having a severe HDFN outcome is approximately 5–6%. More specifically, considering that there is a 96% overall survival rate for fetuses affected by maternal antibodies using modern antenatal treatment techniques including intrauterine transfusions [69], the overall rate of maternal alloimmunization and fetal demise as a result of those antibodies is effectively less than 1%. A hospital or emergency service that is contemplating the implementation of an LTOWB program must balance these risks of adverse clinical outcomes against the benefits of using D+ LTOWB for all trauma patients, including females of childbearing age. For males or females who are no longer of childbearing potential, becoming D alloimmunized is of minimal clinical consequence.

Whole blood units collected in CPD are stored between 1 and 6 °C for up to 21 days (depending on the jurisdiction), ideally in a refrigerator in the emergency department or the trauma bay so that they are readily accessible early in the resuscitation. Units can also be stored in validated coolers for transportation in emergency vehicles. When the LTOWB program was initially implemented at the University of Pittsburgh, the units were manually rocked at each nursing shift change, but this practice was stopped when the data indicated that this manipulation was not necessary to maintain PLT function and may lead to increased hemolysis late in the storage period [70, 71]. It has been recently demonstrated that end-over-end rotation produces superior PLT counts at various WB storage time points compared to non-rotated units, with PLT yields ranging between 71 ± 15% and 76 ± 10% of the baseline concentration between 10 and 22 days of rotated storage, with only a 49 ± 12% yield for 12-day-old WB units that were not rotated [72]. Perhaps, the PLTs adhere to the bag or to each other if the WB unit is not rotated, thereby reducing the yield. However, both the percent recovery of the PLTs and the predicted PLT survival once reinfused to the autologous donors were not significantly different between the rocked and unrocked WB units suggesting that not rocking the WB does not affect their survival upon transfusion. In this study, the rocked WB units produced PLTs that were predicted to survive for between 0.8 ± 0.3 and 1.3 ± 0.3 days depending on the storage time [72], which is similar to the 33.7 ± 14.7 hour survival that was found in an earlier study of 7-day-old cold-stored apheresis PLTs that were reinfused to the autologous donor [28]. Some centers reclaim stored whole blood units if they have not been transfused by day 14

and manufacture them into RBC units that can then be transfused up to day 21 or longer if the WB is stored in an adenine containing solution, thereby reducing wastage [39].

Leukoreduction of LTOWB Units

The decision to leukocyte reduce whole blood units for transfusion should consider regulatory requirements that may differ by jurisdiction and the proven benefits of LR (lower rates of alloimmunization, febrile transfusion reactions, and cytomegalovirus transmission) against the potential detrimental effects of LR on the hemostatic potential of the stored whole blood unit. To date, the literature has not demonstrated a clear morbidity or mortality benefit of transfusing leukoreduced red blood cells in the trauma setting: in two randomized controlled trials (RCT), leukoreduced red blood cells did not reduce the rate of infections, organ dysfunction, mortality, or lung complications among transfused trauma patients [73, 74], and in two retrospective studies, LR also failed to improve mortality and a variety of morbidity markers such as organ dysfunction and hospital and ICU length of stay [75, 76], although in another retrospective study LR was associated with a reduction in all types of infections including nosocomial pneumonia [77]. In vitro data have shown that non-leukoreduced WB units stored between 1 and 6 °C for 14 days or more retain their soluble procoagulant factor activity levels, except for factors V and VIII, which are known to be labile during storage [25, 78]. On the other hand, LR with a PLT-sparing filter caused a significant reduction in hemostatic function as measured by thromboelastography (TEG) and thrombin generation assays, especially early during storage, compared with non-LR WB units although other markers of PLT function were unchanged following LR [71]. A recent study has also shown that the choice of LR filter affects the hemostatic properties of stored WB units; WB units that were leukoreduced with a PLT-sparing filter naturally contained more PLTs and had relatively normal TEG tracings for up to 14 days of storage compared with units that were leukoreduced with a non-PLT-sparing filter, which demonstrated grossly abnormal TEG parameters [79]. Screening tests of coagulation and factor activity levels were not significantly affected by the type of LR filter. Similar findings were also reported in an earlier study of PLT functionality measured by TEG and soluble coagulation factor levels in WB that was filtered using a non-PLT-sparing leukocyte filer vs. unfiltered units [80].

Is Transfusing LTOWB Safe?

One of the largest hurdles involved with the implementation of a whole blood resuscitation program is the fear of a hemolytic transfusion reaction following the administration of blood containing ABO-incompatible plasma. One of the first studies of whole blood use in the civilian trauma setting was a randomized pilot trial of leukoreduced ABO-identical WB compared to standard, fixed ratio component therapy in trauma patients [81]. As the WB was leukoreduced using a filter that did not spare the PLTs, for every six WB units transfused, a single apheresis PLT that was stored at room temperature was also administered. The WB in this study was stored for a maximum of 5 days. In both the intent to treat and per-protocol analyses, there were no differences in mortality between the groups. Furthermore, there were no differences in the rate of complications between the WB and conventional component groups, nor was the length of hospital stay or the number of ventilator-free days different between the treatment groups. While this study did not answer the question of the serological safety of transfusing WB with incompatible plasma, these findings indicated that the provision of ABO-identical WB that was leukoreduced, PLT-depleted, and up to 5 days old was clinically safe for civilian trauma patients.

The aforementioned trial excluded group B and AB patients as it was not feasible for the blood center supplying the trauma center to provide WB of these ABO groups. It would, however, be desirable to provide WB to all massively bleeding patients regardless of their ABO group, hence the growing adoption of LTOWB. For

logistical reasons, it would also be desirable to be able to have a longer shelf life of the WB. Several studies in the civilian setting that featured the transfusion of leukoreduced, PLT-replete LTOWB that was stored for up to 14 days have demonstrated the serological safety of transfusing this product to massively bleeding trauma patients. The first report was based on 27 non-group O recipients of a median of 1 unit (interquartile range, IQR: 1–2) of LTOWB, where low titer was defined as <50 by saline tube immediate spin [39]. These 27 non-group O LTOWB recipients did not demonstrate clinical or biochemical evidence of hemolysis compared to 17 group O recipients of a median of 1 unit (IQR: 1–2) of LTOWB; group O recipients are not at risk of ABO-incompatible hemolytic reactions from the transfusion of LTOWB. There was a minor exception of a significantly higher median total bilirubin among the non-group O recipients compared to the group O recipients on the day that the LTOWB was transfused: the median total bilirubin in these patients was still within the normal adult range at that hospital, and by the next day, there was no longer a statistical difference in this parameter between these two groups of recipients. The authors of this report concluded that the transfusion of 1–2 units of LTOWB was safe and proceeded to increase the maximum number of units first to 4 and then to 6 per patient, still with no laboratory or clinical evidence of hemolysis among the non-group O recipients compared in the aggregate to the group O recipients [40, 41] (M. Yazer, June 2019, "personal communication").

There is also an extensive safety record of transfusing group O whole blood in the military setting. In 1952, during the Korean War, over 600,000 units of LTOWB (titer <256) were transfused to combat casualties. Patients typically received 10–30 units of this product, and only four patients were noted to have post-transfusion hemoglobinuria, the etiology of which was uncertain [82]. During an approximately 18-month period during the Vietnam War, 230,323 whole blood units were transfused [83], and only one hemolytic transfusion reaction to a group O unit was reported. This reaction was caused by the accidental transfusion of a high-titer group O whole blood unit to a group A recipient [84]. These experiences highlight the safety of transfusing LTOWB when administered in an intended manner without clerical errors.

Is Transfusing LTOWB Efficacious?

A retrospective study of military casualties who received *fresh warm* WB, which is not a product that is routinely available nor licensed for use in the civilian setting, compared to conventional components demonstrated improved survival among the former group [85]. In the civilian randomized trial of ABO-identical WB mentioned above [81], there were no differences in the primary outcome, the quantity of blood products transfused in the first 24 hours, between the recipients of WB and components in both the intent to treat and per-protocol analyses. There were also no mortality differences between the groups. When patients with severe traumatic brain injury were excluded in a post hoc analysis, significantly fewer blood products were transfused to the patients in the WB group compared to the component group. However, as the WB units were PLT-depleted, it is not clear what role the WB itself might have had in reducing the number of products transfused as all of the PLTs that were transfused in both groups had been stored at room temperature. A study of the thromboelastogram tracings of pediatric trauma patients did not find a statistically significant difference in the maximum amplitude (MA) between patients who received only LTOWB compared to those who received only conventional warm stored PLTs, but clinical outcomes were not measured in this study [86].

Prospective RCTs comparing the use of LTOWB to conventional component therapy in massively bleeding trauma patients are currently underway. One trial is a single-center pilot study of LTOWB transfusion to traumatically injured patients who are transported to the hospital by helicopter, entitled the Pragmatic Prehospital Group O Whole Blood Early Resuscitation Trial (PPOWER; clinicaltrials.

gov identifier: NCT03477006). This American trial's primary outcome is 28-day all-cause mortality in patients who receive two units of LTOWB in the prehospital setting along with up to four more LTOWB units once the patient arrives at the hospital versus those who receive that standard of care for prehospital resuscitation followed by fixed-ratio blood component resuscitation in the hospital. The results of this trial are expected in late 2021.

Another RCT that is in the advanced stage of planning is the STORHM trial (Sang Total pour la Réanimation des Hémorragies Massives), which will employ a non-inferiority design to compare LTOWB to conventional blood components transfused in a 1:1:1 ratio in severely bleeding trauma patients. The primary endpoint will be a thromboelastographic parameter (maximum amplitude, MA) assessed at the sixth hour after admission. Secondary endpoints will include early and overall mortality, lactate clearance (a reflection of the effectiveness of resuscitation), and organ failure at 24 hours post-admission. This trial will begin recruiting 200 patients at six French trauma centers in the second half of 2019.

Until the RCTs are completed, the best evidence for the outcomes of civilian LTOWB recipients comes from retrospective studies. One of the most compelling studies used propensity score matching to compare the outcomes of 135 trauma patients who received a median of 2 units of LTOWB to 135 matched trauma patients who were resuscitated with conventional components [87]. This study found that none of the outcomes, including kidney injury and a variety of mortality measures, were worse among the LTOWB recipients, and in fact, there was a trend toward a faster correction (a median of >5 hours faster) of an elevated lactate level after receipt of LTOWB and perhaps toward lower mortality as well. Furthermore, a study of 18 pediatric trauma patients who were resuscitated with LTOWB found that by using LTOWB, all three blood components were administered more quickly than when the individual components had to be ordered and infused separately despite the availability of a massive transfusion protocol from the blood bank at this hospital [88].

Summary

In civilian medicine, the use of whole blood as a therapeutic blood component for the resuscitation of traumatic patients has until recently been avoided in favor of component therapy. However, there is expanding evidence that the use of LTOWB is a safe and effective intervention for emergency transfusions where aggressive resuscitation is required in the treatment of acutely hemorrhaging patients. Whole blood provides all of the components of blood in a convenient package that is easy to store and transport, and its use should be expanded from the trauma population to other massively bleeding patients where replacement of RBCs, plasma, and PLTs is desirable such as many patients with a postpartum hemorrhage or patients bleeding in the operating room. The successful use of LTOWB to date demonstrates the necessity of the ongoing randomized control trials to determine the efficacy and safety of its use in the resuscitation of the massively bleeding patient.

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Adjunct Factor Replacement

25

Jared R. Gallaher, Christopher M. Freeman, and Martin A. Schreiber

Introduction

Globally, trauma is a major cause of morbidity and mortality. Nearly a billion people sustain an injury requiring medical evaluation with more than five million deaths annually [1, 2]. Although the single leading cause of death after trauma is severe traumatic brain injury (TBI), uncontrolled bleeding is the leading cause of death among the remaining non-TBI patients and the leading cause of preventable death overall [2, 3]. Obtaining surgical control of bleeding is the foremost goal when caring for this population. Following large-volume hemorrhage, many patients develop a derangement of hemostasis, as well as dilutional and consumptive coagulopathy which can result in "medical bleeding," i.e., blood loss that cannot be controlled with sutures or direct compression. In addition, as many as one-third of all bleeding trauma patients present with coagulopathy upon hospital admission [4–6]. Development of early trauma-induced coagulopathy (TIC) is associated with increased mortality and contributes to the "lethal triad" of coagulopathy, hypothermia, and acidosis which can have a mortality as high as 50% [7, 8]. TIC is a well-recognized multifactorial condition resulting from a combination of bleeding-induced shock, tissue injury-related thrombin–thrombomodulin complex generation, and the activation of anticoagulant and fibrinolytic pathways [9, 10]. Early recognition of TIC is the first step in intervening in this high-risk population. This chapter is focused on the role of fibrinogen and prothrombin complex concentrates (PCC) in the management of trauma-induced coagulopathy.

Fibrinogen Replacement Therapy

Fibrinogen is a soluble glycoprotein synthesized in the liver and is the final component in both the intrinsic and extrinsic clotting cascades forming the insoluble protein fibrin [11]. Fibrinogen is cleaved by thrombin to fibrin monomers which are polymerized and stabilized by Factor XIII to form fibrin clot [12]. Fibrinogen also plays a critical role in the aggregation of activated platelets through glycoprotein IIb/IIIa receptors [12]. With fibrinogen playing a central role in the formation of stable clots and platelet aggregation, the role of fibrinogen supplementation in patients with TIC is based on fibrinogen levels reaching critically low levels earlier than any other coagulation protein. Hyperfibrinolysis, as well as the subsequent

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hypofibrinogenemia, occurs as a result of blood loss, consumption, dilution, acidosis, and hypothermia [13–20]. This is supported in the literature where an association between hypofibrinogenemia and worse outcomes (higher risk of requiring massive transfusion and higher mortality) has been reported [19–22]. With the literature demonstrating fibrinogen reaching critically low levels early and hypofibrinogenemia leading to worse outcomes, supplementation has long been considered. Clinical data from surgical patients have shown that postoperative hemorrhagic tendency is increased when fibrinogen levels are below 150–200 mg/dL [23, 24]. This is further supported by recent data showing that fibrinogen levels below 150 mg/dL were detected in 73% of trauma patients with an initial hemoglobin less than 10 g/dl, in 63% of patients with a base deficit of greater than 6, and in 41% of patients who were hypotensive at admission [21, 25]. Consequently, current guidelines recommend fibrinogen supplementation at plasma fibrinogen levels <1–1.5 g/L or <1.5–2 g/L [26–28].

Fibrinogen supplementation can be provided by transfusion of fresh-frozen plasma (FFP), whole blood, cryoprecipitate, or fibrinogen concentrate [29, 30] (Table 25.1). Current massive transfusion protocols (MTPs) involve the early administration of predetermined fixed ratios of blood products (plasma, platelets, and pRBC); the goal has been to correct what had previously been shown to be inadequate replacement of coagulation factors by increasing the ratio of factors to pRBCs. The PROPPR trial showed that patients receiving transfusion in a higher plasma-platelet-pRBC ratio (1:1:1 vs 1:1:2) were more likely to achieve hemostasis within the first 24 hours although there was no significant difference in 24-hour or 30-day mortality between the two groups [31]. Of note, given the low concentration of fibrinogen in FFP, approximately 2 g of fibrinogen in each liter of plasma, correction of fibrinogen with FFP alone would require a large volume (30 mL/kg) of plasma making fibrinogen replacement through FFP alone clinically impractical. Furthermore, this would increase the risk of transfusion-related complications (i.e., transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and viral transmission) [13]. Data suggests that whole blood has a higher fibrinogen concentration than FFP at 3.2 g/L, but it still requires relatively high volumes for replacement [32].

Table 25.1 Concentration of fibrinogen in products available for replacement

Blood product	Approximate fibrinogen concentration	Volume needed to provide 1 g dose
Fresh frozen plasma	2.5 g/L	400 mL
Cryoprecipitate	15 g/L	95 mL
Fibrinogen concentrate	900–1300 mg per dose	1 vial
Whole blood	3.2 g/L	310 mL

Cryoprecipitate contains a higher concentration (8–16 g/L) of fibrinogen than FFP, but its use is limited for several reasons: fibrinogen concentration is not standardized, ABO crossmatching is required prior to transfusion, and the product must be thawed prior to administration [13, 33]. For these reasons, cryoprecipitate has disappeared from blood banks in most European countries [30]. However, it remains the most common source of fibrinogen replacement in the United States. The PROMMTT study found wide variability in the administration of cryoprecipitate in the ten level 1 trauma centers involved in the study [34]. In those patients that received cryoprecipitate, the median time to transfusion was 2.7 hours, and the majority of patients who died of hemorrhage did not receive cryoprecipitate. In the CRYOSTAT feasibility study, 85% of patients randomized to the cryoprecipitate arm received cryoprecipitate within 90 minutes with a median time to transfusion of 60 minutes resulting in consistently higher fibrinogen levels in the cryoprecipitate arm [35].

Fibrinogen Concentrate

Fibrinogen concentrate has been developed and utilized in European trauma systems due to the aforementioned drawbacks to FFP and cryoprecipitate. Fibrinogen concentrate is produced from pooled human plasma using the Cohn/Oncley cryoprecipitation procedure [36]. The

concentration of fibrinogen is standardized; the product is stored as a lyophilized powder at room temperature and can be reconstituted quickly with sterile water, and infusion volumes are low, allowing for rapid administration without delays for thawing or crossmatching [37]. There are several fibrinogen concentrates that are commercially available; however, the most widely used is Haemocomplettan (CSL Behring, Marburg, Germany), a human pasteurized, highly purified, plasma-derived fibrinogen concentrate [38, 39]. Several studies have evaluated the effects of fibrinogen supplementation with this agent in patients suffering from various forms of congenital or acquired hypofibrinogenemic conditions [27, 40].

As fibrinogen concentrate usage has increased, there has been a growing body of observational evidence to support its use in severe trauma. These studies have shown increased clot strength, reduction in blood loss, and reduced transfusion of allogenic blood products [41–46]. However, these studies are observational or retrospective cohort studies and, as of yet, do not provide high-level evidence to support fibrinogen concentrate in trauma-associated severe coagulopathy.

Among these studies, Schöchl et al. published data including 128 bleeding trauma patients and retrospectively evaluated goal-directed coagulation management using thrombelastometry-guided administration of fibrinogen concentrate together with prothrombin complex concentrate. The observed mortality rate was lower in this intervention group compared with the mortality predicted by the Trauma Injury Severity Score (TRISS) and the Revised Injury Severity Classification (RISC) score [41]. As a follow-up to this study, the group performed a subsequent retrospective study comparing blood product requirements between trauma patients treated with fibrinogen concentrate (median dose: 6 g) and/or prothrombin complex concentrate, but no FFP, and patients receiving only FFP. Those patients receiving fibrinogen concentrate and/or PCC only required significantly less RBC and platelet transfusions than those treated with FFP alone [42]. Innerhofer and colleagues compared patients who received fibrinogen concentrate without FFP ($n = 66$) versus fibrinogen concentrate with FFP ($n = 78$) and found a lower volume of blood products transfused (RBC and platelets) in patients receiving only fibrinogen concentrate [47]. Of note, there was no difference in clinical outcomes. This retrospective study increased the strength of its findings by adjusting for patient severity using propensity scores with the analysis of 28 patient pairs confirming the results.

A retrospective study of 294 trauma patients further evaluated whether administration of fibrinogen concentrate is associated with improved outcomes [48]. Although 6-hour mortality was significantly reduced in the fibrinogen concentrate group, overall mortality was not significantly different between groups. In contrast to other studies, the RBC requirement was not reduced in the fibrinogen concentrate group. The only published prospective observational study in this clinical setting is by Weiss and colleagues [49]. A total of 223 patients were included, of whom 62 (28%) were trauma patients and received fibrinogen concentrate in association with FFP. After a median dose of 12 g of fibrinogen, plasma fibrinogen rose to 2.19 g/L at the end of the surgery, corresponding to a median increment of 0.045 g/L per gram of fibrinogen administered. Three percent of patients sustained thromboembolic complications perioperatively. Interestingly, the authors found that plasma fibrinogen at the end of surgery and 24 hours after administration of fibrinogen concentrate was significantly higher in the survivors compared with the non-survivors. A systematic review of the use of fibrinogen concentrate in trauma patients has been published [50]. Two randomized trials have assessed the role of fibrinogen concentrate in cardiac surgery [50, 51]. In the first trial, involving 20 patients undergoing elective coronary artery bypass grafting, the infusion of 2 g of fibrinogen concentrate significantly reduced postoperative blood loss [51]. In the second more recent study, thrombelastometric-guided intraoperative hemostatic therapy with fibrinogen concentrate was more effective than placebo in controlling bleeding during major aortic replacement surgery [52].

Current Guidelines, Issues, and Future Direction on Fibrinogen Supplementation

Current European guidelines on the management of bleeding and coagulopathy following major trauma recommend treatment with fibrinogen concentrate (or cryoprecipitate) if significant bleeding is accompanied by viscoelastic abnormalities (i.e., rotational thrombelastometry (ROTEM, TEM Innovation GmbH, Munich, Germany) and thrombelastography (TEG, Haemonetics Corp, Niles, IL, United States)) (Grade 1C) revealing a functional fibrinogen deficit or a plasma fibrinogen level of less than 1.5–2.0 g/L [26]. The addition of a fibrinogen level is new from previous guidelines. An initial fibrinogen concentrate dose of 3–4 g is suggested, with successive doses guided by viscoelastic monitoring and laboratory assessment of fibrinogen levels (Grade 2C) [26]. In the United States, fibrinogen is typically replaced utilizing cryoprecipitate administration (each unit of cryoprecipitate contains 150–250 mg of fibrinogen): 1 U/10 kg increases plasma fibrinogen by 50–70 mg/dL [53]. Unlike Europe, fibrinogen concentrate is not approved for patients with acquired bleeding in the United States, and thus, its use in the trauma setting is limited. There is conflicting data regarding cost-effectiveness. An initial study from 2008 showed comparable cost, but a more recent analysis contradicts this finding, noting that the cost of fibrinogen concentrate exceeds the cost of an equivalent dose of cryoprecipitate by $976–$1303 [54, 55]. To be cost-effective, fibrinogen cost must decrease by 44% or reduce ICU length of stay by 0.25–0.66 days. In the same study, cost (in addition to off-label usage and insufficient evidence) was the most common reason among US transfusion fellowship directors for not using fibrinogen concentrate.

To provide more head-to-head randomized data to determine the superior method to replace fibrinogen, the Fibrinogen Early In Severe Trauma StudY (FEISTY, NCT02745041) has begun to enroll patients. This is a multi-institutional randomized controlled trial comparing fibrinogen concentrate to cryoprecipitate using thrombelastometry (ROTEM®) parameters in patients with severe traumatic hemorrhage [56]. Primary outcomes will be (1) time to administration of fibrinogen supplementation with either fibrinogen concentrate or cryoprecipitate and (2) effect of fibrinogen supplementation on fibrinogen levels. Secondary outcomes include blood product transfusion requirements, thromboembolic complications, hospital length of stay, and mortality. The study will take place in four major trauma centers in Queensland, Australia, and has been recruiting since October 2016. No preliminary results have been released. An additional study, albeit in cardiac surgery patients and not trauma patients, that is ongoing is the FIBrinogen REplenishment in Surgery (FIBRES) trial [57]. This is a multicenter RCT in adult cardiac surgical patients who have clinically significant bleeding and acquired hypofibrinogenemia; patients will receive either 4 gm of fibrinogen or 10 units of cryoprecipitate (dose-equivalent). The results of these trials—FEISTY and FIBRES—will hopefully bring clarity to the debate between the use of fibrinogen concentrate and cryoprecipitate.

Clotting Factor Replacement Therapy

Prothrombin Complex Concentrates

Over the last decade, guidelines have changed substantially for the management of patients on anticoagulation with serious or life-threatening hemorrhage. Consequently, inactive prothrombin complex concentrate (PCC) has become a vital component of oral anticoagulation reversal, especially warfarin [58]. PCC was originally developed as a source of factor IX for the treatment of patients with hemophilia B [59, 60]. It is produced with inactive factors in both three- (II, IX, and X) or four-factor (II, VII, IX, and X) concentrates, with a final overall clotting factor concentration approximately 25 times higher than in normal plasma [61, 62]. The inactive PCC formulation available in the United States is a



Fig. 25.1 The prothrombin complex concentrate (PCC) available in the United States

four-factor product that contains factors II, VII, IX, and X, along with proteins C and S, antithrombin III, and heparin that act as stabilizing agents [63, 64] (Fig. 25.1).

In trauma- or surgery-associated bleeding, either PCC or fresh frozen plasma transfusion along with IV vitamin K is standard practice for warfarin reversal [58]. PCC has the advantage of being a low-volume medication without the need for crossmatching or thawing. Retrospective data from the early 2000s suggested that PCC rapidly reversed INR in patients on warfarin [65–68]. This was further demonstrated by evidence from prospective, randomized studies. The first in 2013, showed non-inferiority for four-factor PCC compared to plasma transfusion in the achievement of effective hemostasis at 72.4% in the PCC group and 65.4% in the plasma group [69]. However, PCC was superior in rapid INR reduction, defined as an INR ≤1.3 at 30 minutes after the end of infusion, which was achieved by 62.2% in the PCC group and only 9.6% in the plasma group. A second, randomized noninferiority trial of similar patients using four-factor PCC in 2015 found similar results [70]. Effective hemostasis was achieved in 89.7% of patients in the PCC group and 75.3% of patients in the plasma group. Rapid INR reduction was achieved in 55.2% of the PCC group and only 9.9% in the plasma cohort with a treatment difference of 45.3% (95% CI 31.9, 56.4, $p < 0.0001$). There were no differences in mortality or other outcomes. In 2016, Lancet Neurology published a multicenter, prospective, randomized trial comparing four-factor PCC to plasma transfusion in adult patients with intracranial hemorrhage related to vitamin K antagonists [71]. The study was terminated early after only 50 patients were enrolled due to safety concerns favoring PCC. The study's primary endpoint was the reduction of INR ≤1.2 within 3 hours of treatment initiation. Only two patients (9%) reached this goal in the FFP group versus 18 patients (67%) in the PCC cohort giving an adjusted odds ratio of 30.6 (95% CI 4.4–197.9, $p = 0.0003$) favoring the PCC group in INR reduction. In terms of clinical outcomes, no patients in the PCC group died from intracranial hematoma expansion, while 5 out of 23 (22%) died from intracranial hematoma expansion among patients who received FFP.

While these clinical trial data suggest that PCC is superior for INR reversal, questions remain about whether PCC use improves clinical outcomes in patients on warfarin. A 2015 Cochrane review of four randomized trials of PCC use in patients on a vitamin K antagonist concluded that while evidence suggests that INR reversal is faster with PCC and avoids the potential complications of plasma transfusion, there is little quality evidence for improvements in bleeding-associated complications [72]. The review's meta-analysis showed a risk ratio of mortality of 0.93 (95% CI 0.37–2.33) for PCC compared to plasma transfusion and also found no association with a decrease in blood transfusions due to a lack of data. In 2018, a systematic review of both retrospective and prospective data of PCC use in patients with intracerebral hemorrhage had similar conclusions [73]. They reported that despite robust data on INR reversal, there is limited or poor-quality data on both functional outcomes and mortality and that more research is needed.

Previous nonclinical data showed there may be an increased thromboembolic risk when using PCC with corroboration from animal model studies [60, 74, 75]. However, recent clinical data has not supported this concern. A previously referenced 2013 randomized trial of four-factor PCC versus plasma in acute bleeding found a similar

number of thromboembolic events in the PCC and plasma groups with four related thromboembolic events (3.9%) in the PCC group and three in the plasma group (2.8%) [69]. A post hoc pooled analysis of data from two randomized clinical trials comparing four-factor PCC and plasma showed no differences in thromboembolic events between the two treatments with an event occurrence of 7.3% in the PCC group and 7.1% in the plasma group (risk difference 0.2%, 95% CI −5.5–6.0%) [76]. An older meta-analysis of mixed prospective and retrospective data found an even lower prevalence of thromboembolic events in patients who received four-factor PCC with a weighted mean of 1.8% (P5% CI 1.0–3.0) [77]. Currently, the preponderance of the literature does not suggest there is a high rate of thromboembolic complications with PCC, especially in comparison to plasma [78].

PCC dosing may be standardized or based on weight, INR, or both. Observational data have shown that variable dosing based on weight and INR is likely superior to low fixed dosing at reversing INR. A 2012 prospective, observational non-inferiority cohort study showed that fixed dosing was associated with an inferior reversal of INR compared to variable dosing [79]. In 2017, a retrospective historical cohort study comparing the two approaches showed that fixed dosing required an additional dose for an adequate reversal in 32% of patients compared to 8% in the variable dosing group (p = 0.04) [80]. Investigators in the Netherlands are currently enrolling in a multicenter, randomized controlled non-inferiority trial comparing low fixed dose to variable dosing PCC which will hopefully provide more clarity on an optimal dosing strategy with higher quality data [81]. Current dosing guidelines are usually institutional. Lastly, retrospective data suggest that the four-factor formulation of PCC may be superior to the three-factor formulation with similar or better INR reversal efficacy and fewer thrombotic complications [82, 83]. This has clinical relevance especially in countries where both are available or four-factor PCC is not approved.

The role of PCC in the reversal of direct oral anticoagulants (DOACs) is still evolving. Data from animal models have been mixed, but several studies have demonstrated at least a partial reversal of some DOAC agents [84–87]. Human studies involving healthy volunteers have provided some evidence that PCC at least improves coagulation laboratory values, especially in patients who are taking Factor Xa inhibitors and not dabigatran [88–94]. A recent retrospective study of patients receiving PCC for major bleeding showed that effective hemostasis occurred in 80.6% of patients but with no control group [95]. A prospective, observational study in patients with acute, major bleeding associated with rivaroxaban or apixaban achieved “effective” hemostasis in 69.1% of patients but was worse in patients with intracranial hemorrhage [96]. However, these results are difficult to interpret given the high mortality at 32% and the lack of a control group. Lastly, a 2019 systematic review of ten case series with a total of 340 patients concluded that the quality of available data is too uncertain to make conclusions on whether administering PCC is superior to simply stopping a Factor Xa inhibitor at the time of the bleeding event [97]. The pooled proportion of patients with successful hemostasis was at best 77% (95% CI 63–92%), but again, there was no comparison group. Many centers administer PCC to patients taking a Factor Xa inhibitor considering the lack of another option for reversal. This may change over the next few years with the approval of andexanet alfa, a new reversal agent that targets Factor Xa inhibitors, although its high cost and relatively high thromboembolic rate may slow adoption [98, 99]. Randomized, prospective data comparing PCC with andexanet alfa is likely the next step in guiding reversal practice. Ciraparantag, a potential reversal agent that binds to heparin and oral factor Xa and IIa inhibitors, is still very early in development [100].

There is recent retrospective evidence that PCC may be useful in patients with trauma-associated coagulopathy that are not on oral anticoagulation pre-injury. In 2015, a retrospective study showed that in patients not on warfarin with traumatic brain injury who received PCC as an adjunct to FFP transfusion had quicker INR reversal and fewer red blood cell and FFP

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transfusions compared to FFP alone. Their quicker reversal also led to a shorter time to craniotomy [101]. A similar study examining patients with pelvic or extremity fractures found comparable results with quicker INR reversal and shorter times to operative fixation in patients who had trauma-associated coagulopathy [102]. In 2014, the University of Arizona published retrospective data from 2011 and 2012 using propensity score matching that compared FFP alone to FFP plus PCC in patients not on oral anticoagulation pre-injury. They showed quicker INR reversal and fewer red cell and FFP transfusions and demonstrated an improvement in mortality for the FFP+PCC cohort, 23% vs. 28% (p = 0.04) [103]. The same institution repeated the study with patients from 2015 and 2016 and found similar results but with an even greater mortality benefit for the FFP + PCC group at 25% vs. 33% (p = 0.04) [104]. In Europe, its use has been recommended as a second-line agent in the setting of a normal fibrinogen and trauma-associated delayed coagulation on viscoelastic monitoring, but these guidelines acknowledge that its role is still evolving [105, 106]. Previous literature reviews have concluded that there is still insufficient, high-quality evidence to support its use outside of anticoagulation reversal and that its safety in these patients needs to be investigated [107, 108]. So, while there is promising retrospective data on its use outside of anticoagulation reversal, given the lack of robust, prospective evidence, the role of PCC in trauma-associated coagulopathy remains unclear and off-label in the United States.

Additional Factor-Based Therapies

Other factor-based treatments include activated PCC (aPCC) and recombinant activated Factor VIIa (rFVIIa). Neither is currently recommended for routine use in life-threatening bleeding [58]. Activated PCC is available in the United States as FEIBA® (factor eight inhibitor bypassing activity) and was developed initially for the treatment of patients with hemophilia A or B. It contains several inactive factors and a substantial quantity of activated Factor VII [109]. In contrast to inactive PCC, clinical data on its use in patients with severe bleeding that do not have hemophilia is very limited and comes from animal models, healthy volunteers, or very small case series [88, 110–113]. While early experiences are promising, both in terms of INR reversal and the low rate of associated thromboembolic complications, given the relative quality of data for inactive PCC, it is difficult to justify using it in these situations.

Similar considerations apply to the use of rFVIIa. Recombinant Factor VIIa is available as NovoSeven® in the United States and is approved for inhibitor-complicated hemophilia working as a thrombin augmenter [114]. There is more clinical data exploring the use of rFVIIa in situations like trauma-associated hemorrhage, but these results have been mixed. A 2012 Cochrane review of 29 RCTs examining the use of rFVIIa in patients with bleeding without hemophilia concluded that its use outside of patients with hemophilia should be limited to clinical trials [115]. This was due to a lack of evidence that rFVIIa improved mortality or other clinical outcomes and its association with an increase in arterial thromboembolic events with a pool risk ratio of 1.45 (95% CI 1.02–2.05). A recent retrospective, propensity-matched analysis of cardiac surgery patients receiving either rFVIIa or three-factor PCC also raised concerns about the relative risk of both renal failure and postoperative bleeding complications for rFVIIa [116]. Like aPCC, the lack of evidence relative to inactive PCC keeps us from recommending its routine use outside of patients with hemophilia.

Conclusion

Trauma-induced coagulopathy is a life-threatening condition with a high associated morbidity and mortality. This chapter focuses on the role of fibrinogen replacement and PCC. There is little disagreement that in the bleeding trauma patient, fibrinogen must be replaced. However, the source of fibrinogen—concentrate or cryoprecipitate—varies by nation and institution.

Data supporting fibrinogen concentrate are promising, but there is little evidence prospectively comparing it with cryoprecipitate. The results of upcoming RCTs will help guide clinical decision-making in the future. Less uncertainty exists regarding the use of PCC therapy in patients with traumatic coagulopathy which have become the standard of care for the reversal of warfarin-induced coagulopathy. PCC is also used for the reversal of direct oral anticoagulant (DOAC) medications, but its effectiveness is less well understood. Despite this uncertainty, it remains the most available option for the reversal of those DOACs that do not yet have a specific antagonist.

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Anti-fibrinolytics

26

Dominik F. Draxler, Robert L. Medcalf, and Russell L. Gruen

Abbreviations

aPC	Activated protein C
ATC	Acute traumatic coagulopathy
CSF	Cerebrospinal fluid
EACA	ε-Aminocaproic acid
ICH	Intracranial haemorrhage
ISS	Injury Severity Score
LY30	Lysis 30 min after maximum amplitude of the clot assessed with thromboelastography
PAI-1	Plasmin activator inhibitor 1
PAI-2	Plasmin activator inhibitor 2
TAFI	Thrombin-activatable fibrinolysis inhibitor
TBI	Traumatic brain injury
TEG	Thromboelastography
t-PA	Tissue-type plasminogen activator
TXA	Tranexamic acid
u-PA	Urokinase-type plasminogen activator

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The Fibrinolytic System

The fibrinolytic system consists of serine proteases, protease inhibitors and cell surface receptors that orchestrate the spatial proteolytic activation of the zymogen plasminogen to plasmin [1]. Plasmin in turn can act upon various substrates, but the most recognized is fibrin, the structural scaffold of blood clots. Hence the fibrinolytic system is most recognized for its role in fibrin removal and the subsequent dissolution of blood clots.

Under physiological conditions, plasmin generation is facilitated by tissue-type plasminogen activator (t-PA) and to a lesser extent by urokinase-type plasminogen activator (u-PA). In contrast to u-PA, the activity of t-PA is critically dependent on the presence of fibrin. Indeed, this fibrin dependency increases the capacity of t-PA to generate plasmin by more than two orders of magnitude [1]. Both plasminogen and t-PA bind to exposed lysine residues on fibrin (Fig. 26.1, *panel A*) that subsequently promotes plasmin formation on the fibrin surface. When associated with fibrin, plasmin is protected from its major natural inhibitor alpha$_2$-antiplasmin (α_2-antiplasmin). This strict regulation of fibrinolytic activity is not only facilitated by lysine-specific binding and circulating α_2-antiplasmin but also by inhibition at the level of the plasminogen activators. Plasmin activator

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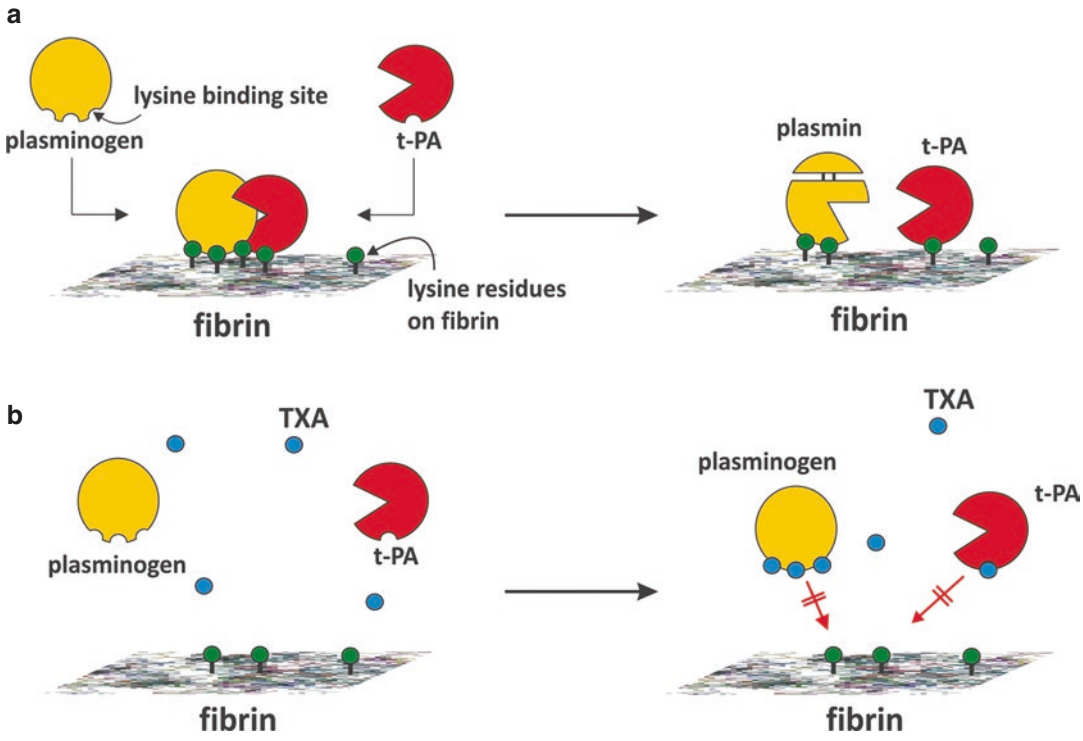


Fig. 26.1 Panel A: plasminogen and t-PA both contain lysine binding sites that bind to lysine residues on fibrin, facilitating the proteolytic activation of plasminogen to plasmin and subsequent degradation of fibrin [1]. Panel B: TXA, a lysine derivate, blocks the lysine binding sites on plasminogen and t-PA, thereby inhibiting their co-localization on fibrin and plasmin generation [6]

inhibitor (PAI)-1 and to a lesser extent PAI-2 are potent circulating inhibitors of both t-PA and u-PA. Finally, an important mechanism of regulation is maintained by thrombin-activatable fibrinolysis inhibitor (TAFI), a carboxypeptidase that by proteolytic removal of lysine residues on fibrin can drastically reduce plasmin generation [1].

In addition to its clearly recognized role in fibrinolysis and clot removal, the plasminogen activating system has now also been implicated, unexpectedly, as having a critical role in the central nervous system (CNS) [2, 3]. It is not the purpose of this chapter to review this newly recognized role, for which we refer the reader to other reports describing the involvement of the plasminogen activating system in various physiological and pathophysiological processes, including the CNS (learning and behaviour, neuronal modulation, blood-brain barrier permeability, addiction), as well as tissue repair, inflammation and metastatic dissemination of cancer cells [4, 5]. What is not known, however, is the extent (if any) to which over-activation of this system (i.e. hyperfibrinolysis) or its inhibition with anti-fibrinolytic agents influences these "non-fibrinolytic" processes.

Hyperfibrinolysis

Hyperfibrinolysis is the accelerated degradation of fibrin, resulting in increased likelihood for bleeding [6]. The first entry mentioning hyperfibrinolysis in PubMed is from 1957 in an article dealing with bleeding disorders of newborn [7]. Hyperfibrinolytic activity has also been described in malignant diseases, such as prostatic cancer [8] and leukaemia [9], as well as infectious diseases [10]. Pathological hyperfibrinolysis was first described by Starzl et al. in 1963 in the context of liver transplantation [11].

Hyperfibrinolysis in Trauma-Induced Coagulopathy

Trauma-induced coagulopathy was long thought to occur due to a simple depletion or dilution of procoagulant factors. However, with the identification of acute traumatic coagulopathy (ATC) [12, 13], it was found that an additional mechanism was based on disturbances in the balance of pro- and anticoagulant factors, platelets, the endothelium and the fibrinolytic system. ATC develops quickly, within minutes after tissue injury and systemic hypoperfusion. Factor V deficiency can be detected, probably due to activation of protein C (aPC), resulting in systemic anticoagulation [14]. The activated vascular endothelium seems to be critically involved in the generation of aPC, which inactivates not only the clotting factors V and VIII but also the potent fibrinolytic inhibitor, PAI-1. As t-PA is also released from endothelial cells under these conditions, it is likely to be more active due to the reduction of PAI-1 activity in the presence of aPC [14, 15]. Nowadays, hyperfibrinolysis is widely acknowledged as being an important participant in the pathophysiology of trauma-induced coagulopathy. It is present in approximately 18% of severely injured trauma patients at hospital arrival. The presence of hyperfibrinolysis is consistently associated with the highest mortality [16–18] as well as death due to exsanguination [19]. Fibrinolysis phenotypes vary and their differences will be discussed in more detail in Chap. 9 [21].

While other chapters in this book have adequately covered the fibrinolytic system in general and the occurrence of hyperfibrinolysis as well as diagnostic evaluation of trauma-induced coagulopathy, this chapter will present an overview of the history and use of anti-fibrinolytic drugs in the treatment of patients with severe trauma, including TBI.

Anti-fibrinolytic Drugs

Two anti-fibrinolytic drugs are currently available—TXA and ε-aminocaproic acid (EACA)—both discovered by Okamoto et al. around 1960 [22, 23]. These agents were first used in the treatment of gynaecological complications as recently reviewed [6]. A third anti-fibrinolytic agent, aprotinin (Trasylol), a direct plasmin inhibitor, first described over 80 years ago was used as an anti-fibrinolytic drug in patients with pancreatitis and in more recent times during cardiac surgery and many other indications associated with bleeding [24]. However, aprotinin was temporarily withdrawn from the market as it was shown to be associated with significantly increased mortality from myocardial infarction, heart failure and cardiogenic shock in patients undergoing high-risk cardiac surgery [25]. However, after further review, the European Medicines Agency lifted the suspension of aprotinin in 2012 (see section below).

TXA and EACA both are analogues of the amino acid lysine. By reversibly binding to lysine binding sites within plasminogen and thereby competing with the binding to fibrin and other targets containing exposed lysine residues, these analogues efficiently prevent plasmin formation on the lysine bearing substrate (Fig. 26.1). It is also important to mention that the interaction of plasmin itself with fibrin depends on lysine binding and is also inhibited by TXA [6].

Tranexamic Acid

TXA is the most widely used anti-fibrinolytic drug and binds to plasminogen 10–16-fold more efficiently than EACA [26]. Its licensed indications for clinical use are broad and vary significantly between different countries. The maximal concentration of TXA in the plasma is reached 15 min after intravenous administration, while the terminal elimination half-life of TXA has been stated to be approximately 2–3 h [6]. Maximal inhibition of plasmin activity ranges from 30 min in plasma to 2 h in muscle and heart referring to data from animal studies [27]. TXA has been reported to sufficiently suppress fibrinolysis at plasma concentrations of 5–10 microg/mL or 10–15 microg/mL [6, 28], and despite a serum half-life of only ~3 h, an adequate inhibition of fibrinolysis can be expected for up to 8 h [29]. This has been further addressed in a recent systematic review of

in vitro and in vivo pharmacodynamics studies [30]. Plasma TXA concentration was 28.7 (21.5–38.5 [8.7–89.0]) microg/mL at approximately 1 h after administering a 1 gram intravenous bolus to prehospital trauma patients [31]. The investigators also described an open two-compartment pharmacological model for TXA with bodyweight being the main covariate. Metabolism in the liver is minimal and excretion happens mainly via the kidney, which implicates the need of dose adjustment in patients with renal impairment in order to avoid toxic accumulation [6].

Dose regimens vary depending on indication as well as the country in which it is prescribed [6]. TXA can be administered by intravenous, intramuscular or intraosseous injection; orally or topically, TXA is stable and can be stored at a wide range of temperatures with a long shelf life. Although no major drug interactions are known, it is often recommended that TXA not be given blindly in combination with thrombin, factor IX complex concentrates or anti-inhibitor coagulant concentrates in order to avoid the risk of thrombotic complications.

In general, TXA is considered a safe drug. Historically the greatest concern has been related to the induction of seizures, because it is capable of crossing the blood-brain barrier. Cases of seizures after TXA administration have been described in particular in patients with impaired renal function [32, 33]. Some studies have suggested that TXA impairs GABA-mediated transmission [34], but it might also relate to the inhibition of non-fibrinolytic effects of plasmin activity on neurons in the brain although this remains speculative. More common adverse effects of TXA are diarrhoea, nausea and vomiting, dizziness, hypotension and allergic dermatitis [32]. In order to avoid hypotension, it has to be administered slowly over 10 min (100 mg/min) [35]. The use of TXA during pregnancy and lactation period is, in principle, possible. However, as it passes the placenta and can also be detected in breast milk, its use should be carefully considered [32]. A recent meta-analysis on the safety and efficacy of anti-fibrinolytics in paediatric surgery found it appears to be safe for use in children [36].

ε-Aminocaproic Acid

EACA is a lysine analogue as well and can be administered intravenously as well as orally [6, 24]. Peak plasma levels after oral intake are reached after approximately 1.2 h. The clearance happens mainly via the kidney and only a small fraction gets metabolized [26, 37]. The plasma half-life of EACA has been stated to be 2 h [24], while 85% of the intravenously injected drug is cleared after 3 h. However, as EACA can reach the entire extracellular space, EACA is likely to be detectable in the urine for up to 36 h. Plasma concentrations of 1 mM sufficiently suppress fibrinolysis [29]. Indications for EACA are similar as for TXA and include cardiac surgery, hepatic cirrhosis, gynaecological complications and also bleeding associated with malignant diseases [37]. EACA, like TXA is a well-tolerated drug, although muscle necrosis has been described after prolonged use, with consequences ranging from mild myalgia to rhabdomyolysis and renal impairment. Caution has been advised for its use if there is evidence of intravascular clotting, in which it should be used only in combination with heparin, and the combination of EACA with factor IX complex concentrates or anti-inhibitor coagulant concentrates might increase the thrombotic risk [37].

Other adverse reactions that have been observed with EACA include bradycardia and hypotension, gastrointestinal problems, seizures, delirium and syncope. Moreover, pruritus and rash, headache, oedema and malaise have been reported [37]. The use of EACA in pregnant women and nursing mothers has not been well studied but, like TXA and EACA, has been used in children without apparent undue harm [36].

Aprotinin

Aprotinin was also widely used as an antifibrinolytic drug and acts by directly inhibiting plasmin activity [6]. Aprotinin has been used in various bleeding conditions, particularly in surgical interventions, such as cardiac surgery [38].

However, after the BART trial was published in 2008 revealing a significant association of aprotinin with death of cardiac causes [25], it was withdrawn from the market. However, in Canada, it was soon re-authorised for use in cardiac surgery as only patients undergoing high-risk surgical procedures were included in the BART trial and mortality rates were clearly higher than in other studies [6, 24]. This led to the conclusion that aprotinin might still be beneficial in non-high-risk cardiac surgery [24]. In order to avoid hypersensitivity reactions, the use of aprotinin is contraindicated if already administered within the last 12 months. In addition to its thrombogenic potential, aprotinin increases the risk of renal impairment [38]. The use during pregnancy has been tested and considered safe based on animal studies [38].

Safety and Efficacy of Tranexamic Acid in Non-trauma Applications

Considerable experience exists with the use of anti-fibrinolytics in elective surgery, especially cardiac and orthopaedic surgery [6]. A meta-analysis conducted by the Clinical Randomisation of an Antifibrinolytic in Significant Haemorrhage (CRASH-2) investigators, in which TXA was compared with no TXA in elective and emergency surgery, concluded that the need for blood transfusion was reduced by one-third if TXA was administered [39] and that this effect exists for all surgical specialties. Uncertainty remained regarding the effect of TXA on thromboembolic events, such as myocardial infarction (MCI), stroke, deep vein thrombosis (DVT) and pulmonary embolism (PE) [39]; however, in the "Aspirin and Tranexamic Acid for Coronary Artery Surgery" (ATACAS) trial, both the safety and efficacy of TXA were demonstrated for cardiac surgery [40]. Furthermore, hypermenorrhoea, post-partum haemorrhage and bleeding due to implanted contraceptives are typical indications for TXA in gynaecology [6]. TXA appears to be particularly effective for treatment of post-partum haemorrhage when given soon after onset of bleeding [41]. The "WOMAN" trial was a randomized, double-blind, placebo-controlled trial of women aged 16 years and older experiencing post-partum haemorrhage after a vaginal birth or caesarean section and was conducted in 21 countries. The trial was originally conceived using a composite outcome of death or hysterectomy. However, investigators found that the decision for hysterectomy tended to be made at the time of randomization, so the study was adjusted to include at least 20,000 subjects to enable adequate power for a primary mortality outcome. Haemorrhagic deaths were significantly different between groups and were reduced with TXA ([RR] 0·81, 95% CI, 0·65–1·00; $p = 0·045$). The difference was also more pronounced if TXA was given within 3 h of giving birth ([RR] 0·69, 95% CI, 0·52–0·91; $p = 0·008$). There were no differences reported in other outcomes including hysterectomy rate or adverse thrombotic complications.

The Use of Tranexamic Acid in Trauma

Despite being recognized for its effectiveness at reducing bleeding in elective surgery, TXA was used little in trauma until publication of the CRASH-2 study [42, 43]. The CRASH-2 study tested the effectiveness of early in-hospital administration of 2 g TXA (as 1 g bolus over 10 min in 100 ml saline, followed by an 8-h infusion of 1 g in 1 litre of saline), versus placebo, on all-cause in-hospital mortality within 4 weeks of injury. The clinical trials network of the London School of Hygiene and Tropical Medicine was mobilized to enrol 20,211 patients in 274 hospitals in 40 countries. Inclusion criteria were broad—adult patients who were assessed by the treating clinician to be bleeding or at risk of bleeding, as indicated by systolic blood pressure of <90 mmHg or heart rate >110 bpm. Drug and placebo were matched, and both patients and staff were blinded to treatment allocation. Secondary outcomes included death due to specific causes and the need/frequency for blood transfusions.

The study found that TXA, compared with placebo, was associated with 9% reduced relative risk of all-cause mortality. Subsequent pub-

lications have reported subgroup analyses, cost-effective analyses and mechanistic hypotheses [44–47]. Of particular importance, the risk of death due to bleeding, a secondary outcome, was temporally related—the earlier TXA was given, the more effective it appeared to be, with 32% reduction, compared to placebo, in relative risk of death due to bleeding if given within an hour of injury and 21% if administered 1–3 h after injury and, surprisingly, a 44% increased risk of death if given >3 h after injury [42]. Subsequent pooled analysis of CRASH-2 and WOMAN trial data confirmed the important effect of drug dose timing on haemorrhagic death, where survival benefit decreases by 10% for every 15 min of treatment delay, until all benefit is lost at 3 h.

The findings of CRASH-2 were widely promulgated with a clear message that all trauma patients who were at risk of bleeding should be given TXA within 3 h of injury. In the United Kingdom, TXA became the first drug to be fast-tracked for use in the National Health Service under the Government's "medicines innovation scheme", and case-based payments in the new trauma system were tied to TXA administration. Some European guidelines were quickly amended to recommend administration of TXA in trauma patients who are bleeding or at risk of haemorrhage as early as possible [48]. Many institutions throughout the world incorporated TXA into their massive transfusion protocols for bleeding trauma patients. The implications for prehospital administration have been widely canvassed [49], and many physician-led prehospital ambulance services have begun to administer it in the field [50], including helicopter emergency medical services [51], and are now tested in these settings in RCTs [52, 53]. A recently completed prospective, observational study comparing 70 trauma patients receiving 1 g TXA on-scene with a matched control group of 38 patients not receiving TXA demonstrated clot stabilization in viscoelastic testing and a reduction in fibrin degradation products with the anti-fibrinolytic [54].

Wafaisade et al. also described an early survival benefit with prehospital TXA (compared with matched patients not receiving TXA), which was largest in patients having severe injury. The authors did not detect any difference with respect to thromboembolism [55].

A retrospective study found a reduction in the requirement for in-hospital blood transfusion and massive transfusion protocol in patients receiving prehospital TXA. Thromboembolic events were not significantly higher in the TXA group [56].

A prospective cohort study on severely injured trauma patients with hyperfibrinolysis on admission confirmed an early (6 h) survival benefit as well as safety with respect to thromboembolic complications of TXA, but did not describe improved long-term outcomes, such as 24-h and 30-day mortality or 24-h RBC transfusion need [57].

The recently published CRASH-3 trial further demonstrated that TXA reduces early (within 24 h) mortality in patients with mild-to-moderate TBI but not in patients with severe head injury (TBI) without enhancing the risk for thromboembolic complications, if administered within 3 h of head injury [58].

The Military Application of Tranexamic Acid in Trauma Emergency Resuscitation (MATTERs) study was a retrospective observational trial published in 2012 [59]. Eight hundred ninety-six patients suffering from combat injury and receiving at least 1 unit of packed red blood cells have been included, of whom 293 were treated with TXA. Despite a significantly higher ISS (25.2 vs. 22.5), the TXA group presented lower mortality rates than the non-TXA group (17.5% vs. 23.9%), with a more pronounced benefit in patients receiving massive transfusion (≥10 unit of packed red blood cells; 14.4% vs. 28.1%). In contrast to the CRASH-2 trial, however, this retrospective study identified a significantly increased rate of thromboembolic events in patients administered with TXA. However, the authors emphasize that higher injury severity of the TXA group has to be taken into consideration which might be associated with thrombotic complications independent of TXA administration [59]. Inconsistent dosing and the lack of standardized indication criteria for TXA treatment are other obvious limitations of the MATTERs trial [60].

A summary of clinical trials performed in recent years in order to explore the use of TXA in trauma patients is provided in Table 26.1.

Table 26.1 Clinical trials investigating the use TXA in trauma patients

Published trials	Study design	Mortality	Blood products	Thromboembolic complications	References
CRASH-2	International, prospective, randomized, double-blind, placebo-controlled multicentre trial investigating the effect of TXA in trauma patients who were bleeding or at risk to bleed	TXA administration was associated with a reduction in RR of all-cause mortality of 9% and with a RR reduction of death due to bleeding of 21% if administered within 3 h after injury. However, later administration was associated with an increase in RR for death of 44%	No difference in the need for blood products, RR 0.98 (0.96–1.01), $p = 0.21$	No difference in vascular occlusive events, RR 0.84 (0.68–1.02), $p = 0.084$	Shakur et al. [43] Roberts et al. [42]
MATTERs	Retrospective observational study investigating TXA administration vs. no TXA in patients with combat injuries who received at least one unit of packed red blood cells	TXA was associated with decreased mortality (17.4% vs. 23.9%) in patients receiving at least one unit of packed RBC, with a pronounced effect in individuals receiving massive transfusion (14.4% vs. 28.1%)	Received by all study patients	Significant increase in pulmonary embolism (2.7% vs. 0.3%, $p = 0.001$) and deep venous thrombosis (2.4% vs. 0.2%, $p = 0.001$)	Morrison et al. [59]
Valle et al. [35]	Retrospective study comparing treatment with TXA vs. no TXA in a subset of the most severely injured ICU patients	In a population of most critically injured patients referring to ISS, TXA was detrimental, regardless of what time post-trauma it was administered and associated with increased mortality (27% vs. 17%)	No difference (96.7% vs. 96.7%)	–	Valle et al. [35]
Cole et al. [73]	Prospective single-centre cohort study investigating the use of TXA in severely injured patients with or without shock	TXA was independently associated with a decrease in MOF (OR, 0.27) and adjusted all-cause mortality (OR, 0.16) in shocked patients	No difference	No difference	Cole et al. [73]

(continued)

Table 26.1 (continued)

Published trials	Study design	Mortality	Blood products	Thromboembolic complications	References
Harvin et al. [72]	Retrospective single-centre study assessing the effect of TXA treatment in trauma patients with diagnosed hyperfibrinolysis	TXA administration was associated with increased unadjusted mortality (40% vs. 17%). After multivariate regression, it was found to be an independent risk factor for 24-h mortality, but not for in-hospital mortality	Significantly increased transfusion requirements in the TXA group	No difference in thromboembolic events (6.3% vs. 4.4%, $p = 0.389$)	Harvin et al. [72]
Wafaisade et al. [55]	Propensity score-based matching of primarily admitted trauma patients who were treated with TXA during the prehospital phase with patients who had not received prehospital TXA	Early survival benefit with prehospital TXA, which was largest in patients with severe injury load	No difference in transfusion requirements (massive transfusion, 5.0% vs. 5.8%, $p = 0.85$)	No difference with respect to thromboembolism (5.6% vs. 8.3%, $p = 0.58$)	Wafaisade et al. [55]
Moore et al. [67]	Prospective single-centre cohort study	In patients with physiological fibrinolysis, TXA predicts mortality after adjustment for the Injury Severity Score	Patients requiring massive transfusion and given TXA received more red blood cells ($p = 0.036$), plasma ($p = 0.003$) and cryoprecipitate ($p = 0.026$) within 24 h of injury	–	Moore et al. [67]
Stein et al. [54]	Prospective, multicentre, observational study assessing the coagulation status in 70 trauma patients receiving TXA (1 g intravenously) on-scene versus a control group of 38 patients previously published without TXA	–	Prehospital TXA results in clot stabilization in viscoelastic testing and a reduction in fibrin degradation products	–	Stein et al. [54]

Table 26.1 (continued)

Published trials	Study design	Mortality	Blood products	Thromboembolic complications	References
Khan et al. [57]	Prospective cohort study on patients with admission hyperfibrinolysis (on viscoelastic test), treated with or without TXA, propensity score matched in 1:2 ratio for demographics, admission vitals and injury severity	Patients with hyperfibrinolysis on admission treated with TXA had an early (6 h) survival benefit No improvement in 24-h and 30-day mortality	Transfusion in 24-hunits, median [IQR] RBC 12 [7–22] vs. 15 [10–33], $p = 0.11$ Plasma 10 [3–16] vs. 15 [7–22], $p = 0.03$ Platelets 10 [6–18] vs. 13 [7–19], $p = 0.13$ No improvement in 24-h RBC transfusion need	No increase in the risk of thromboembolic complications DVT 3.2% vs. 6.5%, $p = 0.59$ PE (asymptomatic) 3.2% vs. 0%, $p = 0.55$ PE (symptomatic) 3.2% vs. 3.2%, $p = 1.00$ Stroke 3.2% vs. 3.2%, $p = 1.00$	Khan et al. [57]
El-Menyar et al. [56]	Retrospective study comparing matched adult trauma patients receiving or not receiving prehospital TXA	No significant difference in mortality	Reduction in the requirement for in-hospital blood transfusion and massive transfusion protocol	Thromboembolic events were not significantly different	El-Menyar et al. [56]
Myers et al. [74]	Retrospective single-centre study evaluating the effects of TXA on venous thromboembolism in propensity score-matched patients, adjusted for prespecified confounders	No significant survival benefit	TXA group had significantly higher transfusion requirements	Threefold increase in the risk of venous thromboembolism	Myers et al. [74]
CRASH-3	International, prospective, randomized, double-blind, placebo-controlled multicentre trial investigating the effect of TXA administration within 3 h (until 2016 8 h) after TBI	Among TBI patients treated within 3 h of injury, the risk of head injury-related death was reduced with TXA compared with placebo (12.5% vs. 14.0% in the prespecified sensitivity analysis that excluded patients with a GCS score of 3 or bilateral unreactive pupils at baseline) Mortality was reduced in mild-to-moderate, but not severe, head injury	–	The risk of vascular occlusive events was similar in the TXA and placebo groups (RR 0.98 (0.74-1.28))	CRASH-3 Coll. [58]

TXA tranexamic acid, *RR* relative risk, *OR* odds ratio, *IQR* interquartile range, *ISS* Injury Severity Score, *MOF* multiple organ failure, *GCS* Glasgow Coma Scale

Unresolved Issues in the Use of Tranexamic Acid After Trauma

Editorials questioned whether CRASH-2 provided sufficient evidence for widespread adoption in civilian and military settings [61]. The question whether the addition of TXA to the complex management regime currently practised in advanced trauma systems is effective and efficient is still being tested in ongoing trials. However, the results from two additional randomized placebo-controlled clinical trials (e.g. the WOMAN trial and CRASH-3) have provided further evidence to support the early use of TXA for acute bleeding.

The interactions of TXA with age-related comorbidities and pharmacotherapy are not well understood and certainly require further investigation [61]. Furthermore, the ideal dosage regimen and possible interactions with other drugs, such as fibrinogen concentrates, prothrombin complex concentrates and recombinant activated factor VIIa, are still unknown and will hopefully be revealed by ongoing prospective trials [62]. Severe conditions like multitrauma and shock can lead to impaired renal function within 1 h, as demonstrated in animal experiments [63], which certainly has to be considered regarding dose adjustment in order to avoid toxic accumulation of TXA [62]. A recently performed pilot trial compared two different dosing schemes and placebo in patients undergoing cardiac surgery with cardiopulmonary bypass [64]. There was no difference in fibrinolysis (defined by the authors as lysis 30 min after maximum amplitude of the clot (LY30) of ≥7.5% assessed with TEG), and outcome has been described between the two TXA groups and placebo despite the distinct difference of a 30 mg/kg bolus followed by 16 mg/kg/h infusion in the high-dose group and a 5 mg/kg bolus followed by 5 mg/kg/h in the low-dose group. A significant increase in D-dimer levels, however, was detected in the placebo group in comparison with both TXA groups. The authors stated that a higher number of cases will be necessary to clearly identify differences in fibrinolysis and outcome. In order to determine the optimal dose of TXA in trauma patients, clinical trials also testing different dosing regimen are underway [53].

Another question that still needs to be clarified is why early TXA administration shows a beneficial effect, but late administration was associated with a detrimental effect *due to bleeding* in the CRASH-2 trial. It has been speculated that the differential effects of TXA in relation to time after trauma observed might be due to PAI-1-mediated suppression of fibrinolysis and the conversion of the early coagulopathy into a disseminated intravascular coagulopathy with thrombotic phenotype (i.e. disseminated intravascular coagulation can manifest as bleeding) [46, 65]. This notion is supported by a recent study indicating that 60% of a severely injured patient population actually present with fibrinolysis shutdown as determined with TEG [19]. The authors hypothesized that fibrinolysis shutdown might be the "missing link" in post-injury venous thromboembolic complications. They furthermore stated that haemorrhagic shock might be the promoting factor for hyperfibrinolysis, whereas tissue injury and subsequent PAI-1 release could be the cause for fibrinolysis shutdown, and suggested that TXA should be administered according to viscoelastic test guidance [66]. The same authors suggested, in a later prospective cohort study enrolling 232 severely injured trauma patients admitted to their trauma centre, that in patients with physiological fibrinolysis, TXA predicts mortality after adjustment for the Injury Severity Score [67].

Interestingly this observation is further supported by a retrospective observational single-centre study, conducted by Valle et al., exploring the effects of TXA administration to patients with severe trauma [35]. Patients included were subjected to emergency surgery at arrival at the hospital, and TXA-treated patients were compared to patients not administered TXA by nearest neighbour matching assessed with propensity scores involving sex, age, mechanism of injury, TBI, transfusion requirements, systolic blood pressure and Injury Severity Score (ISS). Those in the TXA group received 1 g of TXA as a bolus followed by an infusion of another gram over 8 h. Surprisingly, regardless of the administration

time after injury, TXA was found to be associated with increased mortality and increased requirements for total fluid, packed red blood cells and fresh frozen plasma, compared with patients not administered with TXA. The authors therefore supported the use of TEG in order to identify those trauma patients who suffer from hyperfibrinolysis and would hence most likely benefit from an anti-fibrinolytic therapy. Using this technique, LY30 of more than 3% has been determined to represent relevant hyperfibrinolysis and to predict the need for anti-fibrinolytic therapy [68, 69]. However, some disagree with the necessity for viscoelastic clot assays in this regard [70]. Referring to the CRASH-2 trial where TEG has not been used [43], they argue that the time required to conduct those assays would result in an unnecessary delay for patients in receiving TXA, given that earlier use was also shown to be more beneficial [70]. Nevertheless, it has to be mentioned in this regard that TXA did not significantly reduce the necessity of blood products in the CRASH-2 trial [43]. This could possibly be explained by a presumably large proportion of patients without relevant fibrinolytic activity. Indeed, subgroup analysis showed that the beneficial effect of TXA was most prominent in the group with severe shock, as defined by a systolic blood pressure <75 mmHg with respect to all-cause 28-day mortality [42, 71]. Like Valle et al., a study by Harvin et al. also did not find a survival benefit of TXA treatment after trauma [72]. In this retrospective single-centre study, TEG was performed on 1032 trauma patients with hyperfibrinolysis (LY30 > 3%). Of those, 10% received TXA (1 g as bolus followed by another gram over 8 h) and 90% did not. Significant baseline differences between TXA and non-TXA groups were also described, whereby individuals of the treatment group were older (37 vs. 32 years median age), were more severely injured (ISS 29 vs. 14), had lower systolic blood pressure (103 mmHg vs. 125 mmHg) and presented higher base excess (5 mmol/L vs 2 mmol/L) indicating shock. The unadjusted mortality was significantly higher in the TXA group (40% vs. 17%). The authors acknowledged the obvious selection bias resulting from the study design, and after multivariate regression adjusting for sex, age as well injury severity, they found that TXA was not an independent predictor of in-hospital mortality but rather of 24-h mortality. Yet, no differences between the two groups were observed with respect to thromboembolic complications. However, the authors themselves indicated that this was not a definitive study and further studies will be required to substantiate these findings.

A recent study by Cole et al. compared TXA with no TXA in severely injured patients (ISS > 15) with or without shock admitted to the ICU [73]. Shock was defined by a base deficit of ≥6 mEq/L. Patients in the TXA group received 1 g as loading dose within 3 h after injury, followed by 1 g over 8 h. In this prospective cohort study, the authors identified TXA treatment to be beneficial with respect to mortality and multiple organ failure outcome in patients with shock. However, no significant benefit was observed in the cohort without shock, yet, also no increased risk of thromboembolic events has been reported.

Another retrospective study described an (threefold) increase in the risk of VTE and suggests a personalized treatment strategy [74].

Further data strengthening the efficacy and safety of TXA as presented for severe trauma in CRASH-2 and more recently from CRASH-3, however, comes from the WOMAN trial, which has demonstrated a mild yet significant reduction in death due to bleeding in post-partum haemorrhage patients treated with TXA [41].

In order to explain the contradictory effects of TXA treatment with respect to time of administration after injury, it also remains a possibility that TXA treatment may have a counterintuitive effect at promoting bleeding by *promoting* fibrinolysis. This unexpected consideration is based on the fact that u-PA-mediated plasminogen activation is actually promoted by TXA [75]. While TXA does indeed inhibit fibrinolysis initiated by t-PA or u-PA on the fibrin surface, a different scenario occurs in solution. This is because the binding of TXA to kringle domains in plasminogen in the solution phase conformationally alters plasminogen in a manner that renders it more open to u-PA-mediated activation; hence, TXA can therefore promote plasmin formation when u-PA is

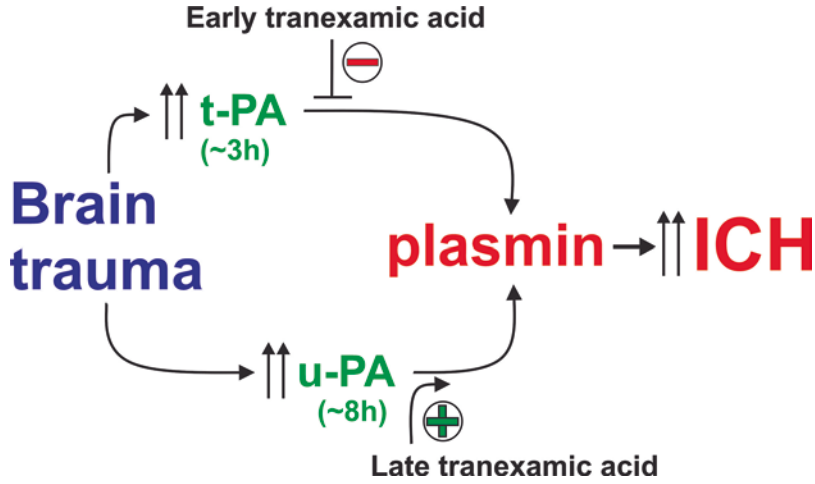


Fig. 26.2 TXA inhibits t-PA-mediated, but enhances u-PA-mediated, plasmin formation. CSF levels of t-PA peak ~3 h, while those of u-PA ~8 h post-TBI, as shown in mice [47]. (TBI traumatic brain injury, ICH intracranial haemorrhage)

present. A similar observation was made by Urano et al. in 1988 where EACA was shown to promote the activation of t-PA in the solution phase [76].

A recent publication investigating the role of the fibrinolytic system in a mouse model of TBI revealed that both t-PA and u-PA levels were increased in the cerebrospinal fluid (CSF) after TBI (Fig. 26.2), but there were marked differences in the temporal profile of each protease: t-PA levels increased rapidly (within 3 h) and returned to baseline by ~8 h [47]. In contrast, u-PA levels increased later peaking at ~8 h. Moreover, administration of TXA immediately after TBI resulted in reduced haemorrhage in this mouse model, consistent with its blocking effect against t-PA-mediated fibrinolysis. In stark contrast, administration of TXA 8 h post-TBI, where u-PA levels were maximal, resulted in an *increase* in haemorrhage, reminiscent of the CRASH-2 trial. This finding, albeit in mice, is consistent with the notion that the increase in deaths due to bleeding in severe trauma patients administered with TXA after 3 h from injury as seen in the CRASH-2 trial may be due to acceleration of u-PA-mediated plasminogen activation, the very process that TXA was thought to stop [77]. Continuous plasmin generation may subsequently lead to a2-antiplasmin consumption further enhancing the fibrinolytic potential of plasmin formed by u-PA [78]. Hence, an interesting question now would be to determine the time course of u-PA activity in cerebrospinal fluid and in plasma of patients following severe trauma. If an increase in u-PA can be detected in patients with severe trauma, future anti-fibrinolytic approaches should be used that target both t-PA- and u-PA-mediated plasmin formation. Indeed, this also raises the prospect of whether aprotinin could be reconsidered in the trauma setting given that it directly blocks plasmin activity, regardless of the activation of plasminogen by either t-PA or u-PA.

In summary, randomized prospective trials have found TXA to be efficacious for decreasing haemorrhagic deaths when given within 3 h of onset of bleeding in three separate bleeding situations, including trauma, post-partum haemorrhage and traumatic brain injury. In each case, there is a lack of evidence for increased adverse events, including thrombotic complications, making TXA the only safe drug currently available for use in a wide range of bleeding situations. Smaller and mostly retrospective studies have attempted to clarify particular clinical settings where TXA may not be useful or may even be harmful. Clearly, after 3 h of injury, TXA should be considered on a case-by-case basis and is likely best given in a goal-directed fashion based upon direct evidence of ongoing hyperfibrinolysis.

What Other Effects Do Anti-fibrinolytic Drugs Have?

Given the broad implication of the fibrinolytic system in various physiological and pathophysiological processes, notably in the CNS, it might be worthwhile to ask what else could be affected by TXA administration. For example, inhibition of the blood-brain barrier permeability associated with plasminogen activation [79] might further contribute to the preventive effects of bleeding, particularly in TBI [5]. The plasminogen activating system is also involved in wound healing [80], which could potentially become impaired by TXA. The wound-healing capacity after trauma is obviously important; however, with dosing regimens suggested so far, pointing to a short-term use rather than a prolonged use of this drug, the effect on wound healing might not be a major concern.

The effects of TXA on the inflammatory response have already been elucidated in cardiac surgery [81]. This phenomenon is also being addressed in the setting of trauma by some of the currently running clinical trials [53, 82]. As the need for red blood cell transfusion was not significantly different between TXA and placebo group in the CRASH-2 trial, it has been speculated that the anti-inflammatory effects of TXA might be involved in the favourable outcome [71].

Furthermore, a benefit of TXA via activating the cellular immune response and reducing infection rates in (non-diabetic) patients undergoing cardiac surgery has been described [83]. This enhanced immune activation on the other hand appears not to be associated with an increased risk of self-reactivity as a precursor for autoimmune disease in a murine model of TBI [84]. Interestingly, with reference to the previously mentioned TXA paradox, it could be demonstrated ex vivo that the effects of TXA on inflammation may also be dependent on the predominant plasminogen activator, whereby complement C5a generation was significantly reduced by TXA in t-PA-mediated fibrinolysis yet enhanced in u-PA stimulated plasmin formation [85].

Interestingly, TXA not only inhibits the generation of plasmin and its ability to bind to fibrin but also blocks binding of α2-antiplasmin to plasmin [6]. In animal models, it was shown to increase thrombus formation and thrombus weight in a dose-dependent manner [86]. Reports on indications other than trauma have described a significant increase in thromboembolic complications in TXA treatment of subarachnoid haemorrhage [87], as well as a non-significant trend in surgery after hip fracture [88].

Controversy and Discussion

Without a doubt, the findings of CRASH-2 and now the CRASH-3 trials are compelling. However, concerns about the applicability of those results for different subgroups of trauma patients have been raised by several authors [49, 68, 71]. It has been suggested that a prospective randomized study performed in a controlled environment with laboratory monitoring of coagulation and standardized transfusion protocols before TXA become standard care in trauma [61].

Moreover, some recommend the proof of existing hyperfibrinolysis before the application of TXA [68, 71]. Yet, the authors of the CRASH-2 study highlight that TXA might also be of benefit in trauma patients with fibrinolytic activity considered normal [20, 46] and hence, it would be necessary to identify those patients who are at risk of bleeding rather than to search for hyperfibrinolysis [46]. Furthermore, it has been suggested that TXA should be used only in severe haemorrhagic shock, when blood pressure levels drop below 75 mmHg [71]. The CRASH-2 investigators, however, argue that early administration was shown to be most efficient and therefore waiting for such a severe condition would be an unnecessary delay [46]. Furthermore, recent guidelines in the United Kingdom have advised against thromboelastography as a reason to withhold anti-fibrinolytic treatment [89].

There was also a suggestion to use TXA only in adults [71]. Children were not included in

CRASH-2, as it is logistically difficult and the fixed dose was preferable to the body weight-adjusted dose necessary for children [46]. However, referring to the ability of TXA to reduce bleeding complications in paediatric surgery, it has been suggested to apply the CRASH-2 study results also in paediatric trauma. An adult TXA dose could thereafter be used in children over 12 years of age, and weight adjustment should be done for younger children [46].

The CRASH-2 authors argued that generalization of results is widely possible, as differences in patient characteristics do not interfere with the pharmacological mechanism of the drug [46]. Other authors have questioned whether concomitant aggressive haemostatic resuscitation in modern trauma systems where preventable mortality is low and incidence of trauma-associated VTE is high might alter the relationship between risk and benefit [49]. Studies in advanced trauma systems have, as yet, not demonstrated the same clear benefit of CRASH-2 [35, 72].

Ongoing Trials Addressing Unresolved Issues

Several studies are currently performed or in the process of planning, aiming to address the open questions. For summary of these trials, please see Table 26.2. The Resuscitation Outcomes Consortium (ROC) is a clinical trial network providing infrastructure and support for research on cardiorespiratory arrest and severe traumatic injury [90]. After CRASH-2 had been published, the ROC committee also suggested a randomized, double-blind, placebo-controlled trial to further elucidate the effect of TXA on TBI. This will evaluate the neurological outcome after 6 months and the 28-day mortality as well as other recovery-related information such as hospital-free, intensive care unit-free and ventilator-free days, etc. Additionally, thromboembolic events and seizures will be assessed in both the TXA and the placebo group.

The "Pre-hospital Anti-fibrinolytics for Traumatic Coagulopathy" (PATCH) trial, another

Table 26.2 Clinical trials planned or currently conducted to address the open questions regarding the use of TXA in trauma patients

Ongoing/ prospective trials	Study design	Open questions addressed	References
ROC	Prospective, randomized, double-blind, placebo-controlled trial exploring the effect of TXA on TBI	Is TXA administration beneficial for TBI patients?	ROC report [90]
PATCH	Prospective, randomized, double-blind, placebo-controlled multicentre trial investigating the early, mainly prehospital use of TXA in severely injured patients, likely to suffer from ATC	Is there a significant net benefit of TXA treatment for patients in countries with advanced trauma care? How does TXA influence parameters of ATC?	Mitra et al. [52]
STAAMP	Prospective, randomized, double-blind, placebo-controlled multicentre trial exploring the effects of TXA administered during air medical transport in trauma patients at risk of bleeding	What is the best dose regimen for the use of TXA in trauma patients? How does TXA administration affect parameters of coagulation and inflammation?	Brown et al. [53]

TXA tranexamic acid, *RR* relative risk, *TBI* traumatic brain injury, *ATC* acute traumatic coagulopathy

randomized, prospective multicentre study, is currently being conducted to further explore unresolved questions, such as the relation of TXA benefit to parameters of ATC [52, 61]. Severely injured patients, likely to have ATC, will be included, and the effectiveness of early TXA administration (prehospital) will be evaluated in developed trauma systems to improve 6-month outcomes and assess adverse effects when used with current management regimes [52].

The "Study of Tranexamic Acid During Air Medical Prehospital Transport" (STAAMP) trial, a randomized, placebo-controlled, double-blind multicentre trial, is underway [53]. Patients will be included if they are between 18 and 90 years old and within 2 h after injury and present with a systolic blood pressure <90 mmHg and a heart rate >110 bpm. TXA or placebo will administered as a bolus of 1 g followed by an infusion of another gram over 8 h. The study is aiming to further investigate the effect of TXA administration during air medical transport in patients at risk of bleeding after trauma. Different dose regimens will be tested pre- and in-hospital, and 30-day mortality will be evaluated. This study will also assess the effects of TXA on coagulation and inflammation post-injury.

Moreover, the "Tranexamic Acid Mechanisms and Pharmacokinetics in Traumatic Injury" (TAMPITI) trial, another RCT, will assess the effects of TXA on immune parameters and drug levels at various time points between 0 h and 72 h post-injury, as well as the incidence of thromboembolic events, seizures and all adverse events in three different treatment arms, including two doses of TXA (TXA 2 g, TXA 4 g and placebo) [82].

Concluding Remarks

The plasminogen-activation system is broadly involved in physiological and pathophysiological processes in the human organism. With respect to trauma, its inhibition could possibly be beneficial not only due to the prevention of haemorrhage but also due to inhibition of blood-brain barrier disruption and subsequent brain oedema as well as its inflammatory properties [5], but this remains to be formally evaluated. TXA is likely to be beneficial, but others have argued for more trials to confirm a net benefit in advanced trauma care systems [49, 72] and to identify which trauma patients should or should be considered for anti-fibrinolytic therapy [91]. The negative effects of TXA later than 3 h after injury observed in the CRASH-2 trial [42] limit the therapeutic window. This may be explained by a rise in u-PA levels at later time points post-TBI (referring to an animal model of TBI and evaluated in CSF), as u-PA-mediated plasmin generation is enhanced by TXA [47, 77] (Fig. 26.2).

Nonetheless, despite the controversies in the clinical literature and the growing appreciation of the broader effect of the fibrinolytic system in physiology, the use of anti-fibrinolytic agents in the setting of trauma has advanced substantially in the past 5 years. While the CRASH-2 trial initially set the stage for the use of TXA, the outcome of the latest series of trials evaluating TXA in trauma is eagerly awaited.

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Ratio-Driven Massive Transfusion Protocols

27

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Abbreviations

ABC score	Assessment of Blood Consumption score
ACS TQIP	American College of Surgeons Trauma Quality Improvement Program
ARDS	Acute respiratory distress syndrome
DCR	Damage control resuscitation
FWB	Fresh whole blood
LR	Ringer's lactate
LTOWB	Cold stored low titer group O whole blood
MODS	Multiorgan system failure
PAMPer	Prehospital Air Medical Plasma trial
PROMMTT	The Prospective, Observational, Multicenter, Major Trauma Transfusion Study
PROPPR	Pragmatic, Randomized Optimal Platelet and Plasma Ratios Trial
RBCs	Red blood cells
SBP	Systolic blood pressure
THOR	Trauma Hemostasis and Oxygenation Research Network
TRALI	Transfusion-related acute lung injury
UOP	Urine output
WFWB	Warm fresh whole blood
WWI	World War I

The Birth of Transfusion

William Harvey first described the circulation of blood in 1628 as "the beat of the heart and arteries, the transfer of blood from veins to arteries, and its distribution to the body." Through experimentation with animal vein ligation, he noted "two kinds of death, failure from a lack [of blood flow] and suffocation from excess" [1]. This led to a few scattered reports of experiments involving blood transfusion in subsequent years, including those of Richard Lower, who performed experiments on dogs with exsanguinating hemorrhage at Oxford, England, in 1666 [2]. However, it would be another two centuries before the understanding of circulation would result in the first recorded human blood transfusion and with it the potential to save people from exsanguinating hemorrhage.

In 1817, during a time when bloodletting to cure disease was rampant, John Henry Leacock questioned, "what is there repugnant to the idea

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of trying to cure diseases arising from an opposite cause [as exsanguinating hemorrhage] by an opposite remedy, to wit by transfusion?" [3, 4]. A year later, the hope of survival in severe hemorrhage was introduced by the British obstetrician James Blundell. After experimenting with blood transfusion in dogs, he performed a series of human blood transfusions for women who bled during childbirth, with a 50% success rate [5, 6]. Over the next half century, reports of transfusion are scarce, with only a handful appearing in the literature. These include transfusions by Samuel Choppin at Charity Hospital in 1854, Daniel Brainard in Chicago in 1860, and US Civil War doctors who transfused at least four Union soldiers [3]. Blood transfusion as the treatment for exsanguinating hemorrhage remained rare due to limited donors, no ability to store blood, and a lack of an effective method of transfusion. As a result, saline intravenous fluids became the standard of care in severe hemorrhage [7].

There was a resurgence of interest in blood transfusions in the 1870s and 1880s with the development of multiple transfusion instruments; however, it was not until the discovery of blood groups by Karl Landsteiner and Reuben Ottenberg early in the twentieth century that reduced transfusion reactions and decreased morbidity and mortality became possible [3, 6, 8, 9]. In 1915, Richard Lewinsohn discovered that sodium citrate with dextrose was a safe preservative, thus enabling blood storage [2, 6].

The Era of the World Wars

The outbreak of World War I (WWI) in 1914 led to a large cadre of severely injured combatants, and the shortcomings of crystalloid resuscitation for hemorrhagic shock became evident. It was demonstrated to result in only a transient response and was ultimately found to be detrimental. Instead, blood transfusions were discovered to be the "most effective means of dealing with cases of continued low blood pressure, whether due to hemorrhage or shock" [10]. The British Expeditionary Force, as well as some American Expeditionary Force hospitals, used whole blood transfusions for resuscitation, and it is estimated that several tens of thousands of transfusions were performed in 1918 alone, saving many lives [6, 8]. At the end of WWI, the Royal Army Medical Corps deemed that advances in blood transfusions were the "most important medical advance of the war" [11].

Following the Great War, the first civilian blood donor service was established in London by the British Red Cross in 1921, employing volunteers to promptly donate fresh blood when contacted [2]. Subsequently, Bernard Fantus at Cook County Hospital in Chicago established the first blood bank in 1937, with whole blood collected in bottles and stored in a refrigerator for up to 10 days [12]. The use of plasma began in 1936, when John Elliot devised a method to separate plasma from red blood cells (RBCs), hailing plasma as a blood substitute for hemorrhagic shock [13, 14]. In WWII, whole blood as well as plasma was used as the primary treatment of hemorrhagic shock [2]. In 1945, Henry Beecher published his recommendations for continued treatment of traumatic hemorrhage based on experience in WWII. He declared whole blood as the superior treatment and advocated administering plasma to "temporarily sustain blood pressure at a level compatible with life for a limited time" to enable "more time to get whole blood into the patient." He also cited a resuscitation goal of warm skin and "good color," which often occurred at a systolic blood pressure around 85 mmHg, noting increased blood loss from resuscitation to higher blood pressures [15].

Following WWII, the military-built national blood program in the United States collapsed. As the use of crystalloid resuscitation in traumatic shock increased, Beecher published an editorial to reiterate the lessons learned from the World Wars, stating, "for the sake of both civil and military medicine it will be well to review recent practices and their foundations. It will be tragic if the medical historians can look back on the WWII period and write of it as a time when so much was learned and so little remembered" [8, 16].

Component Therapy and an Abundance of Crystalloid

In 1951, Edwin Joseph Cohn invented a "blood cell separator" that employed centrifugal force to separate whole blood into layers of RBCs, plasma, and platelets. Promoting his work and coining the term component therapy, Cohn further popularized the use of blood fractionation and the transfusion of blood product components in lieu of whole blood [2]. Component therapy was hailed as economical, because it allowed maximal use of a limited resource, enabling multiple patients to be treated with each unit of whole blood. In addition, the transmission of hepatitis through plasma was becoming increasingly evident [14, 17]. Component transfusion would soon largely replace whole blood transfusion, in spite of a lack of supporting clinical outcome data to support its use.

In the late 1950s and early 1960s, studies performed in elective surgery patients noted extracellular fluid loss and edema at sites of tissue injury in addition to acute blood loss [18, 19]. To further evaluate these fluid shifts in traumatic hemorrhagic shock, Tom Shires et al. performed a study in dogs, concluding that whole blood should remain the replacement for lost blood in hemorrhagic shock. However, the investigators also stated a "judicious amount" of adjunctive Ringer's lactate (LR) could be of value to replace additional fluid losses [20]. This spawned a resurgence of crystalloid use in surgery and trauma patients in an attempt to replace these losses.

In response to the increased use of crystalloid in hemorrhagic shock, Francis Moore and Shires wrote an editorial asking for a return to "moderation" in the use of crystalloid, stating "no conceivable interpretation of these data would justify the use of such excessive balanced salt solution for early replacement in hemorrhage. Neither is the use of saline solutions meant to be a substitute for whole blood. Whole blood is still the primary therapy for blood loss shock" [21]. In spite of these preeminent surgeons' continued emphasis that acute blood loss should be replaced with whole blood, as well as a continued lack of clinical data supporting the use of fractionated blood, in 1970, the American Medical Association published their recommendation that whole blood no longer be routinely used, stating that patients in hemorrhagic shock should be transfused with RBCs supplemented by balanced salt solutions or plasma expanders, and this became the standard of care [22]. Practice thus shifted to primarily RBC transfusions and an increased use of crystalloid for hemorrhagic shock. Large-volume crystalloid resuscitation became increasingly common during the Vietnam War and subsequently spread to civilian trauma centers, despite a paucity of studies validating its safety and efficacy in treating exsanguinating hemorrhage [23, 24].

In 1976, C. James Carrico and Shires outlined a therapeutic plan for traumatic hemorrhage, including an infusion of 1–2 l of LR until whole blood was available for transfusion [25]. Similar to Moore and Shires editorial in 1967, their recommendations were also interpreted as an endorsement for crystalloid resuscitation in acute blood loss and further popularized large-volume crystalloid resuscitation in the treatment of hemorrhagic shock [23]. Defending the resuscitation strategy of RBCs and large volumes of crystalloid, Shackford et al. performed a study comparing RBCs and crystalloid resuscitation to whole blood and crystalloid, finding RBCs and LR could be used (with an average of 6.5 units of RBCs) without producing coagulopathy [26]. However, others remained hesitant to adopt this strategy, noting those with severe trauma were often thrombocytopenic and coagulopathic. They expressed a concern that RBC and crystalloid resuscitation would result in a further dilutional coagulopathy and continued to propose those in hemorrhagic shock should be given whole blood [27].

In the late 1970s and 1980s, studies touting the benefit of "supratherapeutic resuscitation" appeared in the surgical literature. These papers proposed the benefit of maintaining normal values of systolic blood pressure (SBP), urine output (UOP), base deficit, hemoglobin, and cardiac index in an attempt to optimize tissue perfusion [28–33]. This exacerbated the trend toward large-volume crystalloid administration and further

away from the concepts expressed by Cannon and Beecher based on their experiences during the Great Wars [15, 34]. However, when supratherapeutic resuscitation was studied decades later in a prospective and randomized setting, it was shown to be of no benefit and resulted in increased isotonic fluid infusion, diminished intestinal perfusion, as well as a greater frequency of abdominal compartment syndrome, multiple organ failure, and mortality [35–37].

As studies were recognizing the adverse effects of large-volume crystalloid infusion, a resurgence of interest in traumatic coagulopathy occurred. In 1982, the "bloody vicious cycle," now known as the "lethal triad," was coined, citing a frequent downward spiral in severe trauma involving coagulopathy, acidosis, and hypothermia (Fig. 27.1) [38]. While a large percentage of patients who arrived in the emergency department after severe trauma were already coagulopathic, portending a poor outcome [39–42], large-volume crystalloid exacerbated all aspects of this deadly triad [38]. Volumes of crystalloid greater than 1.5 l result in increased mortality [43]. Hypothermia, exacerbated by the administration of unwarmed fluids, also reduces platelet function, coagulation factor activity, and fibrinogen synthesis [44–46]. Crystalloids high in chloride compound cause acidosis, and as a result, significantly more blood products are required to achieve hemodynamically normal parameters. Acidosis also affects platelet aggregation and significantly impairs coagulation factor activity [46, 47]. As surgeons recognized the lethality of the bloody vicious cycle, ways to prevent and treat it were investigated and adopted.

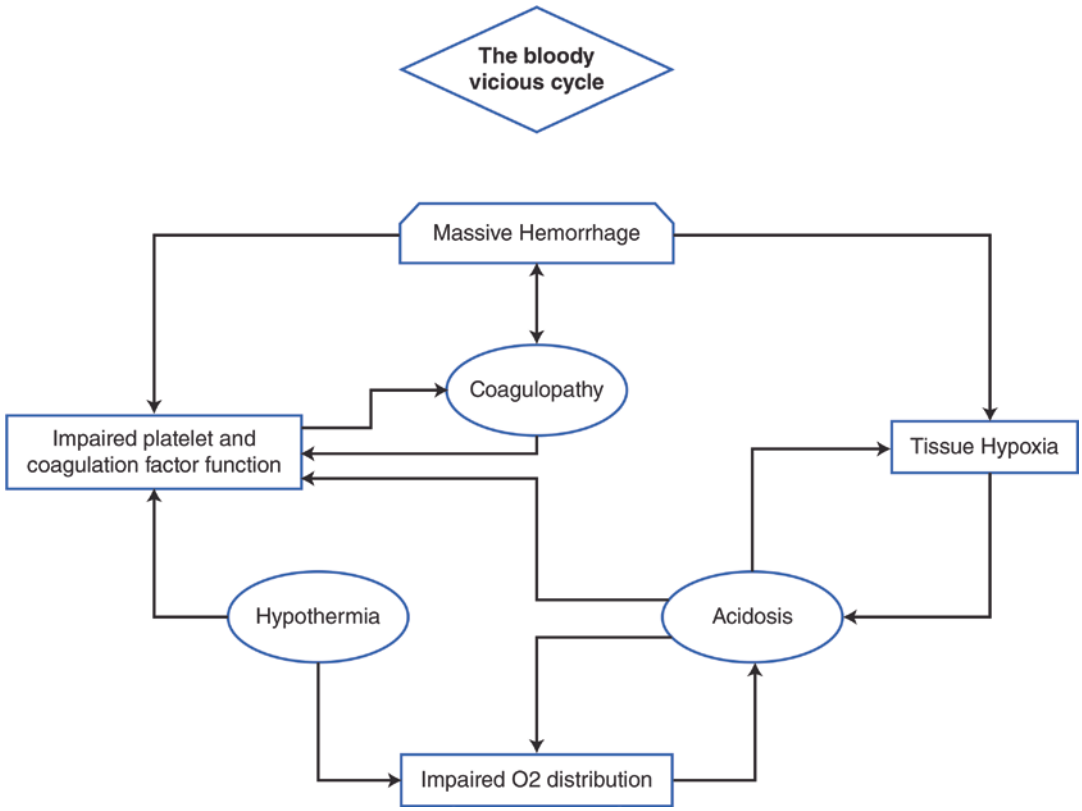


Fig. 27.1 The bloody vicious cycle. (Adapted from Kashuk et al. [38])

Damage Control Resuscitation

The next watershed event in the treatment of severe trauma was the concept of damage control resuscitation (DCR). Returning to the principles of hypotensive and hemostatic resuscitation previously advocated and performed in the eras of WWI and WWII, the focus changed to rapidly controlling hemorrhage, limiting crystalloid use, and infusing blood products to a safe, but lower than normal, blood pressure until operative control of bleeding is established. The protective effect of permissive hypotension prior to hemorrhage control was subsequently confirmed in a randomized controlled trial, which revealed control of bleeding prior to resuscitation to a normal blood pressure resulted in improved outcomes in penetrating and blunt trauma patients [48, 49]. As institutions adopted these strategies, studies investigating DCR protocols revealed a decrease in crystalloid and RBC use, less incidence of the "lethal triad," and improved 24-hour and 30-day survival [50–52].

The Change in Component Ratios: Reinitiation of Plasma and Platelet Resuscitation

In addition to a return to hypotensive resuscitation and prompt surgical control of bleeding, transfusion protocols shifted to include early administration of platelets and plasma in addition to RBCs. This was sparked by studies performed in computer-based simulations, as well as animal studies, that revealed higher ratios of plasma and platelets to RBCs were necessary to prevent and correct coagulopathy in traumatic hemorrhage [53–57]. Moreover, additional beneficial effects of plasma in hemorrhagic shock were being discovered, such as the promotion of vascular stability and restoration of the endothelial glycocalyx, which decrease extracellular fluid loss in shock [58–60].

In 2004, US combat hospitals shifted to an initial massive transfusion ratio of 1:1:1 plasma, platelets, and RBCs. This spawned a retrospective review at a US Army combat hospital in Iraq, which revealed a mortality reduction as the plasma to RBC ratio increased, noting a greater than 50% reduction of mortality in those who received a 1:1.4 ratio of plasma to red blood cells compared to those who received 1:8. This mortality benefit was primarily due to decreasing early death from hemorrhage [61]. Subsequent retrospective and cohort trials were performed in both military and civilian trauma, confirming improved outcomes in those who received an increased ratio of plasma (Table 27.1) [40, 62–70] and platelet transfusions (Table 27.2) [23, 40, 62, 64, 71–73]. In addition, these studies demonstrated the importance of prompt, effective resuscitation, revealing uncontrolled hemorrhagic deaths to occur within 6 hours of injury [52, 61, 64, 74–76].

Table 27.1 Plasma to red blood cell ratios

Plasma to packed red blood cell ratios			
Reference	Type	FFP/RBC ratios	Mortality difference
Borgman et al.	Retrospective	1:1.4 vs. 1:2.5 vs. 1:8	$p < 0.001$[a]
Duchesne et al.	Retrospective	1:1 vs. 1:4	$p = 0.0001$[a]
Gunter et al.	Case-control	≥ 2.3 vs. <2.3	$p = 0.008$[b]
Holcomb et al.	Retrospective	≥1:2 vs. <1:2	$p < 0.01$[b]
Kashuk et al.	Retrospective	1:2 vs. 1:4	$p < 0.001$[a]
Maegele et al.	Retrospective	>1:1 vs. 1:1 vs. <1:1	$p < 0.0001$[c] $p < 0.0001$[d] $p < 0.001$[b]
Scalea et al.	Cohort	1:1 vs. not 1:1	$p = 0.34$[a]
Snyder et al.	Retrospective	≥1:2 vs. <1:2	CI 0.22–0.64[a] With survival bias adjustment CI 0.47–1.5[a]
Sperry et al.	Cohort	<1:1.5 vs. ≥1:1.5	$p = 0.012$[d] $p = 0.2$[a]
Zink et al.	Retrospective	≥1:1 vs. 1:2–1.4 vs. <1:4	$p < 0.001$[c] $p < 0.04$[a]

Adapted from Perkins et al. [62]
[a]In-hospital mortality
[b]30-day mortality
[c]6-hour mortality
[d]24-hour mortality

Table 27.2 Platelet to red blood cell ratios

Platelet to packed red blood cell ratios			
Reference	Type	Platelet/RBC ratio	Mortality difference
Gunter et al.	Case-control	≥1.5 vs. <1.5	$p = 0.001$[a]
Holcomb et al.	Retrospective	≥1:2 vs. <1:2	$p < 0.01$[a]
Inaba et al.	Retrospective	≥1:6 vs. 1:7–1:11 vs. 1:12–1:18 vs. < 1:18	$p = 0.54$ for ≥1:6 vs. 1:6–1:11[b] $p = 0.013$ for ≥1:6 vs. 1:12–1:18[b] $p > 0.001$ for ≥1:6 vs. <1:18
Perkins et al.	Retrospective	≥1:8 vs 1:9–1:16 vs. <1:16	$p = 0.04$ for ≥1:8 vs. 1:9–1:16[a] $p < 0.001$ for ≥1:8 vs. <1:16[a]
Shaz et al.	Cohort	≥1:20 vs. <1:20	$p < 0.01$[c] $p < 0.01$[a]
Zink et al.	Retrospective	≥1:1 vs. 1:1–1.4 vs. <1:4	$p < 0.002$[d] $p < 0.03$[b]

Adapted from Perkins et al. [62]
[a]30-day mortality
[b]In-hospital mortality
[c]24-hour mortality
[d]6-hour mortality

While it became established that increased plasma and platelet transfusions were associated with improved survival, the exact ratio for this effect on mortality remained unknown. Varying ratios of blood products were used in these studies, and they did not agree on whether increased administration of plasma and platelets resulted in an increased incidence of complications, such as transfusion-related acute lung injury (TRALI), acute respiratory distress syndrome (ARDS), and multiorgan system failure (MODS). Also, a limitation of these retrospective studies was highlighted by Snyder et al., who focused on the time it took to obtain and administer varying blood components. Plasma takes time to be prepared and platelets must be constantly agitated during storage; therefore, they are only available to those who survive long enough to receive them from the blood bank. Noting the potential flaw of survival bias inherent in retrospective analysis, this work indicated the likelihood of receiving a high ratio of plasma to RBCs increased with survival time [68].

As a result, the Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) study was designed as a prospective, multicenter trial to further evaluate resuscitation ratios being used at high-volume trauma centers and monitor outcomes. As seen in the previous retrospective studies, hemorrhagic deaths occurred quickly, with 60% of hemorrhagic deaths occurring within the first 3 hours of admission. In spite of clinicians attempting to transfuse a constant ratio, neither plasma nor platelet ratios were consistent across the first 24 hours, and timing of transfusions varied. Thirty minutes after admission, 67% had not received plasma and 99% had not received platelets. Notably, 3 hours after admission, past the time most patients would have already died from hemorrhage, 10% had not received plasma and 28% had not received platelets. However, the study did confirm a survival advantage at 24 hours for higher plasma and platelet ratios early in resuscitation [77].

Subsequently, the Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial was designed to further evaluate the outcomes of frequently used resuscitation ratios. While it did not show an improvement in overall 24-hour survival for a balanced ratio of 1:1:1 (plasma to platelets to packed red blood cells) in comparison with 1:1:2, it revealed a decreased mortality at 3 hours (the timeframe by which most die of hemorrhage) as well as increased hemostasis and decreased death due to hemorrhage at 24 hours (9.2% of death due to hemorrhage when given 1:1:1 versus 14.6% death due to hemorrhage when given 1:1:2). Providing

some allay of concern for increased transfusion complications with increased plasma and platelet administration, this study found there is no difference in the development of ARDS, MODS, venous thromboembolism, or sepsis between the ratios [78]. Further reducing apprehension, recent data has indicated that the incidence of TRALI has decreased with current blood banking practices, and while it still occurs, the rate remains low despite an increase in the use of plasma and platelets in traumatic hemorrhagic shock (1 case of TRALI per 20,000 units of plasma) [79]. Providing further evidence of the value of a return to early platelet transfusion in hemorrhage, a subsequent subanalysis of the PROPPR trial revealed those who received platelets were more likely to achieve hemostasis (94.9% vs. 73.4%) and had significantly decreased 24-hour (5.8% vs. 16.9%) and 30-day mortality (9.5% vs. 20.2%) [80]. As a result of these studies, balanced ratios to approximate whole blood were largely adopted as standard of care, and by 2015, more than 80% of American College of Surgeons Trauma Quality Improvement Program (ACS TQIP) trauma centers targeted a balanced ratio in their massive transfusion protocols [81–83].

Massive Transfusion Protocol Initiation and Practice

Throughout the years, there have been a variety of predictors proposed to identify those in need of massive transfusion. There are risks associated with blood transfusion, including transfusion reactions [84], pulmonary and renal dysfunction [85], and multiorgan failure [86]. Additionally, studies have revealed transfusion does not benefit those who are hemodynamically stable, even in the critically ill and trauma population [87, 88]. In WWII, Beecher used vital signs and "cool skin" as indicators of a need for transfusion [15], and this method continued as studies repeatedly confirmed physical exam and radial pulse could reliably predict the need for intervention [89]. Since that time, there have been a variety of MTP prediction scores, including the Field Triage Score [90], the Shock Index [91], and the Assessment of Blood Consumption score (ABC score) [92]. While these and other proposed algorithms remain important tools [93–96], there remains no universally accepted method for the initiation of massive transfusion, and most MTP activations are based on the provider's experience and an "overall clinical gestalt" to facilitate quick decisions and prompt care [97] (see Chap. 15).

In order to facilitate prompt initiation of blood product resuscitation and decrease preventable death from hemorrhagic shock, massive transfusion protocols and DCR techniques have been adopted to the prehospital arena. When employed, prehospital plasma and RBC transfusion in lieu of early crystalloid resuscitation has shown to prevent coagulopathy and improve early outcomes through earlier initiation of lifesaving transfusion [98–100]. Sparking this important change in the care of severely injured trauma patients in the prehospital setting, Sperry and associates performed the Prehospital Air Medical Plasma (PAMPer) trial in 2018. While most major trauma centers had transitioned to DCR techniques and early balanced resuscitation, the prehospital care lagged behind, with continued crystalloid "standard-care" resuscitation (see Chap. 30). This landmark PAMPer trial examined the efficacy and safety of prehospital thawed plasma in those at risk for hemorrhagic shock (defined as at least one episode of SBP <90 mmHg and HR >108 bpm or SBP <70 mmHg), revealing a lower 30-day mortality (23.2% vs. 33%) and increased hemostasis when plasma was given prior to medical center arrival [99]. A secondary analysis of the study was later performed in 2019, revealing those given red blood cells and plasma to have an even larger mortality benefit and setting the stage for a continued move toward prehospital balanced resuscitation and whole blood transfusion [100].

A Return to Whole Blood

As the benefits of transfusing balanced component ratios became evident in recent years, there has been a push to return to whole blood transfu-

sions for severe traumatic hemorrhage. As Beecher reported in 1945, "men who have lost whole blood need whole blood replacement" [15]. Since 2002, the US military has transfused thousands of units of fresh whole blood (FWB), and published data suggests use of FWB is superior to component therapy, even in a balanced ratio [23, 101–103]. Fresh whole blood resuscitation has shown improved survival compared to component therapy and is known to be a more concentrated, functional product [104]. When component products are recombined in a ratio of 1:1:1 of plasma, platelets, and packed red blood cells, the resulting fluid has approximately a hematocrit of 29%, viable platelet concentration of 8.8×10^8 per liter (around half the platelets of whole blood), and plasma coagulation activity of 65% (Fig. 27.2) [107]. "Thus, during massive transfusion with only blood components given in the optimal 1:1:1 ratio, the blood concentrations for each of the components lead to an anemic, thrombocytopenic and coagulopathic state near the transfusion triggers for each of the components, and administration of any one component in excess only results in dilution of the other two" [4].

In addition, current techniques for the separation and storage of component blood products have been shown to be detrimental and do not prevent a decrease in functionality over time. Plasma coagulation factor activity is known to significantly decline during storage. Thawed plasma can only be stored in liquid form for a maximum of 5 days, because the activity of "labile factors" V and VII is significantly impaired. Liquid plasma only exhibits approximately 35% of factor V activity and 10% of factor VII activity at a storage time of 26 days [108, 109]. Storage temperatures and included preservatives exacerbate hypothermia and acidosis. The longer the blood is banked (especially after 21 days), the worse the resultant acidosis, with a typical 21-day-old unit of RBCs having a pH of 6.3 [110]. In addition, a recent study has indicated current component separation and blood banking practices result in the release of cellular debris in plasma in significant enough quantity to elicit a consequential pro-inflammatory response [111].

Whole blood resuscitation, as used in the Great World Wars, was historically the primary treatment for hemorrhagic shock. The development of blood separation techniques and component therapy was intended to improve blood availability and patient outcomes. However, these advancements, in conjunction with a misguided theory of a need to replace extracellular fluid losses, resulted in the overuse of crystalloid and RBC resuscitation, to the detriment of many patients for decades. In recent years, there has been a return to proven strategies of the past, employing permissive hypotension, prompt surgical control of bleeding, and blood product resuscitation with component ratios approximating the composition of whole blood. Recent conflicts in Iraq and Afghanistan reignited an interest in whole blood transfusion, and in 2014, the US Tactical Combat Casualty Care Committee recommended whole blood should return as the treatment for hemorrhagic shock [102, 112]. Military medicine is subsequently transitioning away from blood product components with a return to warm fresh whole blood (WFWB) and cold stored low titer group O whole blood (LTOWB) [113–115].

In the United States, there remains a fractionated, for-profit blood banking system that largely produces component blood products, and for 40 years whole blood was generally unavailable for the civilian population. Most institutions have a massive transfusion protocol in place that employs a 1:1:1 or 1:1:2 ratio of component transfusion. However, the availability of LTOWB is increasing, and it is being used at more than 20 institutions and prehospital settings across the country [102, 116, 117]. In 2011, the Trauma and Hemostasis Oxygenation Research (THOR) Network was established as an international community of experts in traumatic hemorrhage, promoting continued investigation and improvement in the treatment of hemorrhagic shock. In recent years, there have been multiple advances in the field including improved storage methods such as cold platelets and the reinstitution of liquid and dried plasma, as well as protocol development for the implementation of whole blood transfusion in both prehospital and hospital environments

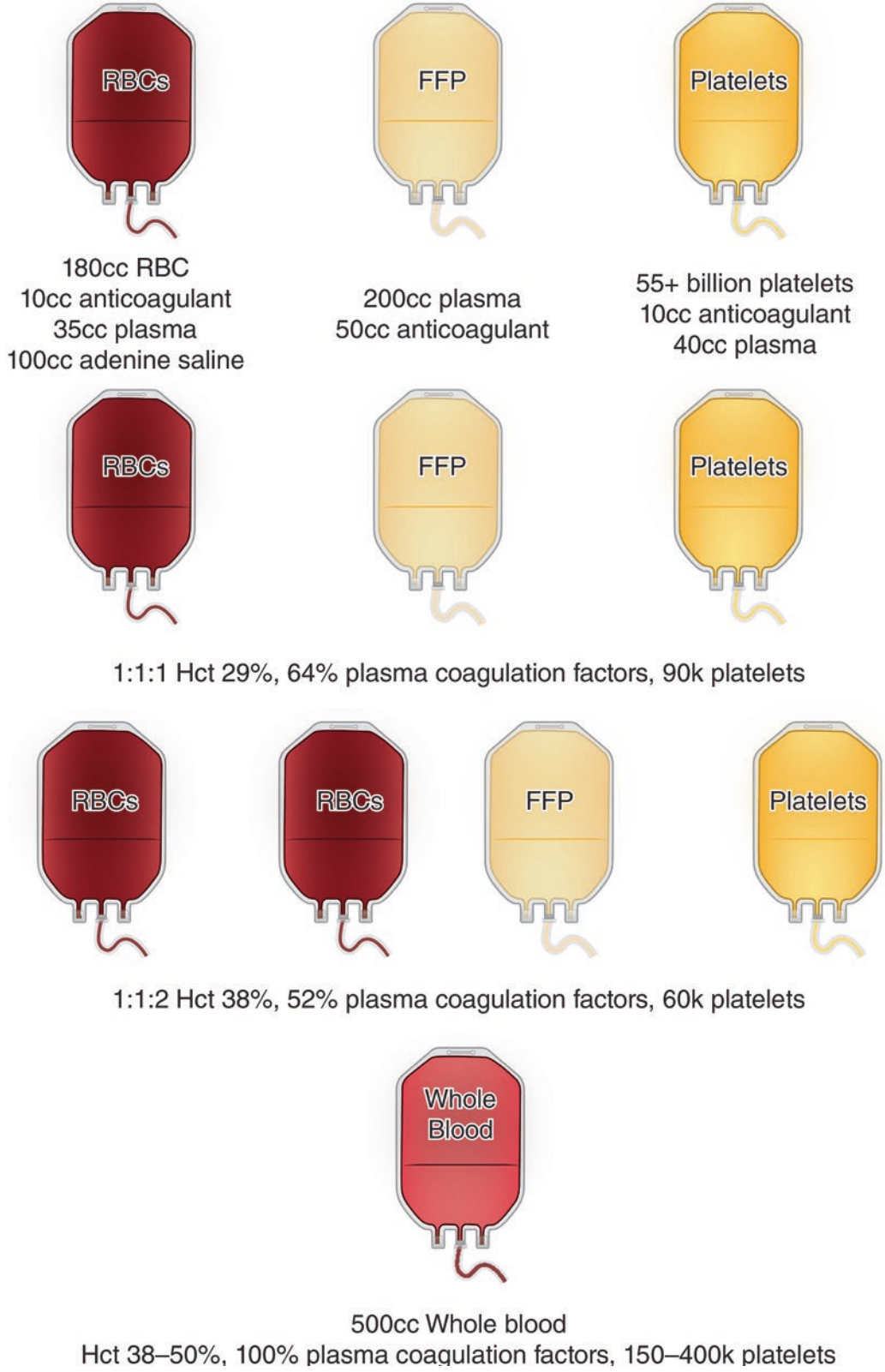


Fig. 27.2 Component ratios. (Adapted from D'Angelo and Dutton [105] and Hess and Holcomb [106])

[118]. Does the future lie in the past [119, 120]? Future studies are needed to determine the efficacy and safety of this pendulum swing, but while many questions remain unanswered, continued inquiry, innovation, and a hope for improved survival in traumatic hemorrhage remain.

Conflicts of Interest There are no conflicts of interest.

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Goal-Directed Massive Transfusion Management

Geoffrey Rogan Nunns, Hunter B. Moore, and Ernest E. Moore

Introduction

Trauma is the leading cause of death under age 40 worldwide and the leading cause of life-years lost under the age of 65 in the United States [1, 2]. Uncontrolled hemorrhage is the leading cause of preventable death following trauma and the overall most common early cause of death, making it the obvious target to reduce mortality [3, 4]. Furthermore, the median time to death from bleeding is <3 hours [5] emphasizing the necessity for prompt intervention for trauma-induced coagulopathy (TIC). On the other hand, blood components have potential adverse physiologic consequences and are expensive; therefore, components should be administered only when needed.

The first use of blood to treat hemorrhagic shock (see "Introduction") was during the Word War I, following the discovery of citrate anticoagulation and ABO blood typing [6, 7]. Because of logistic challenges with whole blood, shock resuscitation was extended to include freeze-dried plasma and albumin in World War II [6]. The work by Shires et al. [8] indicated the need to supplement blood component therapy due to interstitial sodium translocation. Thus, resuscitation in the Vietnam War employed liberal crystalloid in addition to blood transfusions to combat post-traumatic renal failure identified during the Korean War [9]. However, overzealous use of crystalloid led to the development of the acute respiratory distress syndrome during the Vietnam War [10]. After Vietnam, the use of component therapy gained favor in the civilian blood banking systems, with benefits of cost-effectiveness and reduced risk of transmission of infectious diseases [11–13]. Component therapy has the theoretical advantage of allowing transfusion of individualized components tailored to the clinical deficiencies, e.g., RBC given for anemia, platelets for thrombocytopenia, plasma for factor deficiency, and cryoprecipitate for inadequate fibrinogen. Additionally, pathologic clot breakdown or hyperfibrinolysis had specific targeted therapies, such as TXA [14].

As post-injury coagulopathy was recognized and studied, it was initially felt to arise from exogenous factors, notably the bloody vicious cycle of hypothermia, acidosis, and dilutional coagulopathy [15]. This led to the concept of damage control operations with reduced crystalloid use intended to prevent these physiological derangements and the development of post-injury

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coagulopathy [16–18]. However, it was subsequently documented that severely injured patients present with an endogenous coagulopathy (TIC), related to the severity of trauma and depth of shock and ultimately linked to mortality [19, 20]. This is compounded by local clot formation at the sites of injury with factor consumption [21]. The recognition of the varied phases of coagulopathy arising after injury led to the concept of "damage control resuscitation," where preemptive administration of RBC, FFP, and platelets (see Chap. 28) seeks to avert the bleeding tendency and physiologic collapse in this population [21].

Definition of Massive Transfusion

Clinical endpoints are essential to develop diagnostic tools for the management of TIC. Considering the fact that the goal is to prevent death from bleeding, massive transfusion is a rational endpoint. The term massive transfusion was initially defined as patients who received ten units (U) of RBC because it represented twice the normal blood volume [22]. Subsequently, this volume within the first 24 hours of admission was proposed, because earlier time intervals were not available from trauma registries [23]. This definition has been criticized for its survival bias, missing patients who die of bleeding before reaching the threshold of ten units, and understates the importance of the intensity of hemorrhage [24]. Our group originally suggested a threshold of ten units of RBC within 6 hours of arrival because >85% of patients who succumb to bleeding occur within this time frame, while Savage has posited a critical administration threshold of three units of RBC per hour in any hour, and a myriad of other definitions exist [21, 25]. Consequently, we analyzed our prospective trauma activation protocol (TAP) to determine the most accurate definition of massive transfusion, based on volume per time interval [26]. In sum, our data indicates that >4 U of RBCs during the first hour was as accurate as others likely to die from bleeding, and this encompasses the time window in which injured patients die from bleeding. Our data indicates that greater than four units of RBCs during the first hour were as accurate as others likely to die from bleeding, and this encompasses the time window in which injured patients die from bleeding. Subsequently, a review of the PROMMTT database identified >4 U of RBC/h as the optimal definition [27].

Scoring Systems to Predict Massive Transfusion

The definitions of massive transfusion are important to determine the optimal diagnostic test for TIC; however, at the bedside of a critically injured patient, a scoring system is needed to identify the patients at risk of massive hemorrhage (see Chap. 15). Identification and prompt activation of an appropriate massive transfusion protocol is associated with improved survival [28]. An ideal scoring system should be simple and reliant on rapidly assessed metrics, such as physiologic measurements and injury patterns. The ABC score is calculated from four simple components, penetrating injury, positive FAST (Focused Assessment with Sonography in Trauma), arrival SBP <90, or arrival HR >120 [29]. The ABC score is considered positive if two or more criteria are met and it correctly classifies 84–87% of patients. Prehospital scores generally include a physiologic component such as the Resuscitation Outcomes Consortium (ROC) score (SBP of <70 mm Hg or 71–90 with a HR ≥108 bpm), which we use at our institution with the addition of an anatomic component, i.e., mechanism (penetrating torso trauma or pelvic fracture) or a positive FAST that prompts MTP activation [30, 31].

Benefits of Massive Transfusion Protocols

Massive transfusion protocols (MTP) allow a regimented approach to the massively bleeding patient with the goal to improve survival in this population. They allow coordination between the surgical, anesthesia, and blood bank teams to provide rapid administration of appropriate blood

products and avoid waste and overuse. The use of a massive transfusion protocol has been shown to improve blood product utilization, reduce transfusion complications, and most importantly increase survival in trauma patients [32–35]. However, there exist a wide variation in what constitutes a MTP, ranging from the choice of blood products and how they are administered (ratio based or guided based on laboratory assessments of coagulation), together with the use of adjuncts to blood including calcium, crystalloid fluid, and antifibrinolytics [36].

Evidence-Based Massive Transfusion Protocols

Given the wide variation in massive transfusion protocols, our institution has sought to establish a massive transfusion protocol that enables a rapid, efficacious, and appropriate administration of blood products. We sought a balance between hemostatic resuscitation to prevent the development or worsening of TIC while avoiding adverse effects of unneeded transfusions of blood products including transfusion-associated lung injury and multiple organ failure [37, 38].

While many authors support a ratio-based approach that replicates whole blood because it is shown to lead to improved survival [11, 39], this can lead to under- or overtreatment. Principal component analysis has revealed that trauma patients have distinct patterns of TIC that may or may not overlap, including those driven by factor depletion, platelet or fibrinogen depletion, and hyperfibrinolysis [40, 41]. As TIC is not a homogenous entity, the logic of treating it as such with ratio-based protocols is suboptimal. The use of ratio-based strategies (1:1:1) as compared to TEG-guided resuscitation was examined by Tapia et al., comparing the ratio-based strategy to a historical cohort of TEG-guided patients [42]. They examined all patients who received >6 U of RBC in 24 hours from admission and showed that for patients who had suffered a penetrating injury and received >10 U of RBC in 24 hours, the mortality worsened with ratio-based resuscitation (33% vs. 54% mortality).

Rather than use a ratio-based approach, our massive transfusion protocols focus on real-time assessment of the coagulation system in injured patients. The initial tests used to identify TIC (PT/INR, PTT) were not designed for trauma patients but arose to diagnose inherited hematologic disorders and to monitor for anticoagulant dosing [19, 43–45]. Importantly, these tests require 30–60 minutes to produce results and only assess coagulation in platelet-poor plasma, missing crucial metrics of coagulation including platelet function and clot breakdown (fibrinolysis) [46]. The latter limitations of PT/PTT as a measure of coagulation in the trauma patients can be addressed with the addition of measurements of platelet count and function, as well as fibrinogen levels and d-dimer levels to assess fibrinolysis [47]. Platelet count may not reflect the severity of hemorrhage or platelet dysfunction, while platelet aggregometry to assess for function is logistically difficult to implement [48, 49]. Together, we refer to these tests as conventional clotting assays or CCAs.

Given the limitations of CCAs, they would ideally be replaced by a single test that would assess all aspects of the coagulation system and deliver rapid results. This led us to investigate thrombelastography (TEG; see Chap. 20) in the research laboratory [21, 50, 51]. Based on this experience, we were convinced of the merits of TEG, but our hospital administration was reticent. Consequently, we designed a randomized study comparing TEG to CCAs [31] which provided us the evidence to adopt TEG as our standard for TIC management. In preparation for these studies, it is critical to derive thresholds for the TEG measurements to determine specific blood component administration.

Viscoelastic Measurements for Blood Component Therapy

Most believe the current widely used viscoelastic assays (thrombelastography, TEG (see Chap. 20), or rotational thromboelastometry, TEM (see Chap. 19)) are equivalent [52]. As we use predominantly TEG-based resuscitation at our

center, we will focus on its nuances. TEG provides comprehensive assessment of the coagulation system, reporting clot generation and breakdown using whole blood, recreating the conditions experienced in vivo. TEG generates a number of variables, of which we will focus on R-time (or ACT), angle, MA, and percent lysis at 30 minutes. R-time, or reaction time, measures the time to development of a measurable clot and correlates with coagulation factor activity. Angle measured in degrees represents the speed of clot formation and correlates with fibrinogen levels. MA is the maximal amplitude or maximum clot strength and is correlated to platelet function. Finally, clot breakdown is reported at percent lysis at 30 minutes from when MA is reached and when elevated is indicative of hyperfibrinolysis. The standard TEG assay is run as a "native TEG" on whole blood, with a normal R-time of an R-time of 8–12 minutes [53]. However, the use of an accelerant to speed up the initiation phase of clot formation allows a more rapid readout with an ACT available within 5 minutes and an angle and MA within 15 minutes [54]. We use a rapid TEG clinically, which is augmented by kaolin and recombinant tissue factor and phospholipids, activating both the extrinsic and intrinsic pathways [54].

We established a TEG-guided resuscitation protocol and then compared this to conventional clotting assays (CCA) to guide massive transfusions, i.e., PT/INR, aPTT, platelet count, fibrinogen levels, and d-dimers. This trial randomized 111 injured patients, all of whom were randomized to either CCA- or TEG-guided resuscitation [31]. Inclusion criteria were MTP activation and enrolled severely injured patients with a median ISS of 30, of whom 27% suffered penetrating injury. These well-randomized groups showed a decrease in mortality with TEG-guided MTP compared to CCA-guided MTP, with a mortality of 19% in the TEG group compared to 36% in the CCA group ($p = 0.04$). The difference in mortality was predominantly driven by early deaths in the CCA group, largely attributable to hemorrhage (Fig. 28.1). The use of RBC and crystalloid

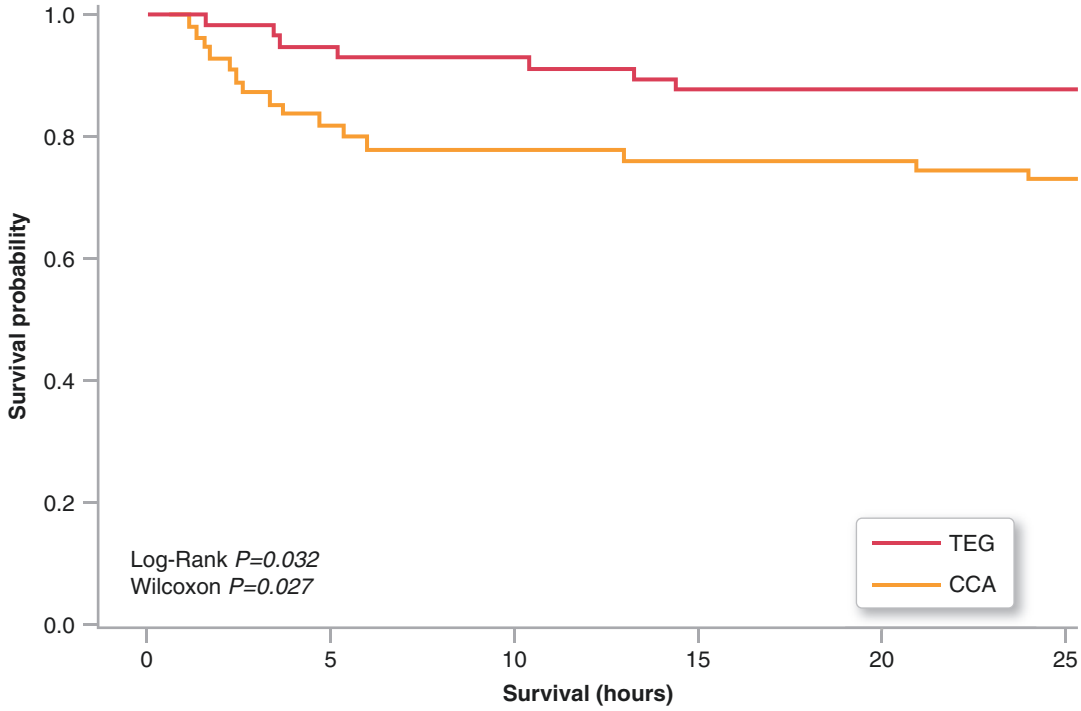


Fig. 28.1 Kaplan-Meier estimates of survival for TEG vs. CCA analyzed as intention to treat, showing a survival benefit in the TEG group [31]

was similar between the two groups (again reinforcing that these were similarly injured groups). Early administration of FFP and platelets (≤2 hours) and overall cryoprecipitate (≤24 hours) were decreased in the TEG-guided group. The survival benefit persisted when patients were examined by both intention to treat and "as treated," as eight patients randomized to the CCA group crossed over to the TEG-guided arm at provider discretion. Additionally, TEG-guided resuscitation resulted in less time in the ICU and fewer ventilator days. Overall, this study demonstrated TEG-guided resuscitation reduces mortality by nearly 50% in patients undergoing a massive transfusion protocol compared to utilizing CCA-guided resuscitation, with less early blood product use. The most recent multicenter randomized trial using ratio-driven resuscitation showed no benefit with 1:1:1 resuscitation over 1:1:2 [55]. The target population in this study was comparable to the Denver TEG trial, and the mortality rates were 22.4 in 1:1:1 and 26.1% in 1:1:2, which are higher than TEG arm (19.6%) in the Denver trial, which was completed during a similar time frame. Therefore, TEG-based resuscitation at our institution remains the standard of care.

Denver Health Massive Transfusion Protocols

The current protocol of massive transfusion in Denver was originally based on thresholds used in Gonzalez et al.'s study [31] which were generated from algorithms to treat bleeding in patients undergoing cardiac surgery. The transfusion thresholds have been modified over time (Fig. 28.2) [56]. This was possible with a large amount of clinical data obtained from routine implementation of TEG in trauma patients at Denver Health with active research and quality improvement to optimize blood product resuscitation [57]. We initiated massive transfusions based on the prehospital vital signs that fulfill ROC/anatomic criteria, with initial delivery of 4 U of RBC and 2 U of FFP. These initial ratios are based on our retrospective data suggesting a survival benefit of a 2:1 RBC/FFP ratio [38] and no survival benefit demonstrated when using higher ratios [55]. With preemptive blood product resuscitation, we believe it is critical to administer calcium with the first unit of blood product, as hypocalcemia is common following severe injury and massive transfusion [58]. Hypocalcemia appears to be exacerbated by citrate, which is used as an anticoagulant in blood products [58]. Further product administration is based on a rapid TEG that is drawn in the trauma bay, with administration of additional products based off the results (Fig. 28.2) [57]. As institutional choices may result in the use of either a different TEG reagent or thromboelastometry, we have established similar guidelines for both kaolin TEGs and ROTEM [59, 60].

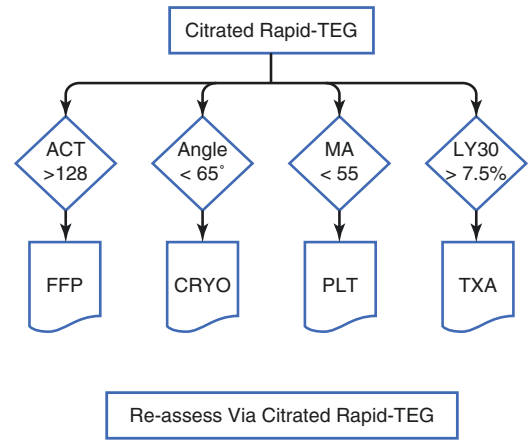


Fig. 28.2 Rapid TEG thresholds for the administration of blood components [56, 57]

Despite the efficiency of TEG-based resuscitation, there are certain scenarios where rapid hemorrhage and lack of results hamper the prompt administration of appropriate blood products. To address this population, we employ our massive transfusion threshold (>4 U of RBC within the first hour of arrival) and administer empiric platelets and cryoprecipitate if there is still ongoing bleeding [50]. Our retrospective review indicated that 65.5% of these patients would have required platelets and 43% would have required fibrinogen.

An important consideration with evolving resuscitations strategies in trauma is to appreciate

the dynamic nature in coagulation following resuscitation. Initial viscoelastic testing will change over time, which can be appreciated within 15-minute intervals [61]. Repeated measures should be conducted while ongoing bleeding is occurring. A recent proposal for quantifying coagulopathic bleeding has been described [62]. An important finding from implementation of this score at our center is that in the emergency department, surgeons do a poor job of predicting who has coagulopathy, while in the operating rooms the scores are highly predictive. Therefore, initial evaluation of all trauma patients at risk of massive bleeding at our center includes viscoelastic testing.

In sum, our ongoing clinical experience and basic investigations indicate that despite the advances made with TEG-guided resuscitation, there are still areas to improve protocols and provide timely administration of appropriate blood products to the bleeding patient. The recently FDA-approved TEG 6S (see Chap. 19) and anticipated ROTEM sigma will provide realistic point-of-care testing, including in the prehospital environment, and thus critical measurements earlier. Considering the increasing use of O negative low-titer whole blood for initial resuscitation (see Chap. 25), the data from these devices should provide timely guidance for the second level of blood component administration.

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Prehospital Resuscitation

29

Andrew-Paul Deeb and Joshua B. Brown

Introduction

While resuscitation of the injured patient has evolved substantially over the last two decades, only recently has it been recognized that prehospital resuscitation can have a significant impact on outcomes. There has been a focus on damage control resuscitation in the hospital-based setting, providing nearly equal ratios of packed red blood cells (PRBC), plasma, and platelets to not only replace oxygen-carrying capacity but also treat trauma-induced coagulopathy (TIC) [1, 2]. However, evidence demonstrates markers of a pro-inflammatory response and coagulopathy are present within minutes of injury at the scene [3, 4]. Thus, the type and volume of fluid that a severely injured patient receives or does not receive in the prehospital setting can set them on a trajectory toward a good or poor outcome.

While initially TIC was thought to be due to dilutional effects of crystalloid, recent work has demonstrated TIC develops with tissue injury and shock independent of resuscitation, shifting the focus toward directly addressing this TIC by restoring coagulation factors and limiting dilutional and pro-inflammatory effects from crystalloid that impair coagulation [5, 6]. Crystalloid remains the de facto resuscitation fluid in the field; however, prehospital blood product resuscitation is becoming more common [7]. This has led to a push to extend damage control resuscitation principles into the field for severely injured patients in hemorrhagic shock.

Resuscitation Access in the Field

Intravenous Access

Prehospital resuscitation begins with obtaining intravenous (IV) access in the field. Because flow rate is directly proportional to the inner cannula diameter and inversely proportional to the length of the catheter, short large-bore IVs are ideal in the bleeding trauma patient. This can be readily achieved with insertion of 16-gauge peripheral IVs. Intravenous access falls within the scope of practice for advanced life support (ALS) providers (i.e., paramedics and advanced emergency medical technicians as well as prehospital flight nurses and advance practice providers). Attempts at peripheral access can prolong prehospital time up to 12 minutes, particularly with failed attempts [8–10]. Thus, transport should not be delayed for attempts at IV access, instead favoring access obtained en route. Current guidelines recommend only two attempts at peripheral IV access prior to moving on to another access modality [11].

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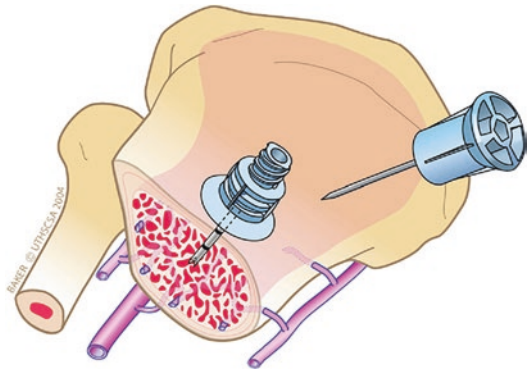


Fig. 29.1 Intraosseous access in the proximal tibia. (Reprinted with permission from Smart et al. [14])

Central venous access is rarely used in the US prehospital system but more commonly performed in European prehospital systems where physicians are routinely providing field care [12]. Some air medical services have the ability to insert central venous catheters in the United States, and studies suggest percutaneous Seldinger technique is preferred for safety and speed of access over cut-down methods [13].

Intraosseous Access

Intraosseous (IO) access has become the favored second-line access modality in most US prehospital systems, owing to its technical ease and speed of insertion with automated drills (Fig. 29.1) [15]. IO placement is commonly performed in the proximal tibia, humeral head, or occasionally the sternum. Further, IO allows infusion of fluids, medications, as well as blood products. Given these characteristics, IO access is often used as first-line access for patients in extremis or cardiac arrest.

Prehospital Crystalloid

Crystalloid Physiologic Effects

Crystalloid is the de facto resuscitation fluid used in the majority of prehospital systems in the United States. Crystalloid is inexpensive, widely available, and highly durable in a range of environmental conditions. Animal experiments in the 1960s and 1970s suggested extracellular fluid compartment deficits that required large volumes of crystalloid to correct in hemorrhagic shock [16, 17]. These findings led to the 3:1 rule of replacement with at least threefold greater volume of crystalloid than estimated blood loss and promulgation of the initial 2 l bolus of crystalloid for prehospital providers.

Subsequent investigation elucidated significant deleterious effects of crystalloid resuscitation, especially in large volumes. Saline in particular can cause metabolic hyperchloremic acidosis which in turn leads to dysregulation of the coagulation cascade enzymes at suboptimal pH levels with impaired thrombin generation. Acidosis from large volumes of saline can also impair cardiac contractility as well as the effectiveness of circulating catecholamines to effect compensatory vasoconstriction [18].

Further, crystalloid fluids incite a pro-inflammatory state with activation of neutrophils, increase neutrophil adhesion, and promote release of tumor necrosis factor-alpha, interleukin (IL)-6, IL-8, and IL-10 leading to intracellular edema and dysfunction, as well as vasodilation and capillary leak [19, 20]. Prehospital crystalloid volume has also been associated with hyperfibrinolysis in some patients, a highly lethal phenotype [21]. Dilutional effects on the coagulation proteins due to crystalloid infusion occur and contribute to clinical coagulopathic bleeding, although they are distinct from the pro-inflammatory effects promoting ongoing coagulopathy in injured patients [5, 18, 19, 22]. These detrimental effects have been borne out in clinical studies demonstrating increased mortality associated with greater volumes of prehospital crystalloid administration [21, 23–25].

Several groups investigated hypertonic saline as a potential prehospital resuscitation fluid, with early promising results [26–29]. Hypertonic saline was hypothesized to have a more favorable physiologic profile as it required a lower volume of fluid to restore intravascular volume and had less pro-inflammatory effects [30–33]. Two large prehospital resuscitation trials were conducted using hypertonic saline compared to isotonic crystalloid [34, 35]. One was performed in

patients with traumatic brain injury (TBI) and one in patients in hemorrhagic shock; however, both were stopped early for futility (Table 29.1). Thus, hypertonic saline has not found use in prehospital resuscitation protocols in the United States.

Prehospital Crystalloid Volume

Given these findings, current practice has moved away from prehospital and early in-hospital resuscitation with large volumes of crystalloid. The landmark trial by Bickell et al. was one of the first to demonstrate withholding crystalloid infusion for patients with penetrating torso trauma until definitive hemorrhage control was achieved significantly improved survival to discharge (Table 29.1) [36]. However, not all studies have reported worse outcomes associated with higher prehospital crystalloid volume, and some have reported improved survival particularly in patients with TBI [37, 48–53]. The question of optimal prehospital crystalloid volume among different patient populations remains. This continues to be an important question, as crystalloid will continue to be the primary prehospital resus-

Table 29.1 Selected randomized prehospital trauma resuscitation trials

Trial	Published	Design and methods	Main result
Bickel et al. [36]	1994	Single-center, randomized patients with penetrating torso injury and SBP <90 to receive immediate crystalloid resuscitation or no crystalloid until surgical control of hemorrhage	8% reduction of in-hospital mortality in delayed fluid group (n = 309) compared to immediate fluid group (n = 289) 30% vs. 38%, p = 0.04
Turner et al. [37]	2000	Multicenter, cluster randomized paramedics (n = 401) to standard crystalloid resuscitation (500 mL bolus with additional crystalloid for signs of shock at paramedic discretion) or no crystalloid resuscitation in trauma patients; paramedics cross over to other resuscitation protocol at trial half completed point	No difference in 6-month mortality in no crystalloid group (n = 699) compared to standard resuscitation group (n = 610) 9.8% vs. 10.4%, p = 0.72
HTS TBI [34]	2010	Multicenter, blinded, randomized patients with GCS <8 and without shock criteria (see HTS shock below) to receive 250 mL of 7.5% HTS + dextran, 7.5% HTS, or normal saline	No difference in 6-month proportion of patients with Glasgow Outcome Scale-Extended ≤4 in HTS + dextran group (n = 359) compared to HTS group (n = 341) or saline group (n = 582) 54% vs. 54% vs. 52%, p = 0.67
HTS Shock [35]	2011	Multicenter, blinded, randomized patients with severe hypotension (SBP <70) or hypotension and tachycardia (SBP 71–90 + HR ≥ 108) to receive 250 mL of 7.5% HTS + dextran, 7.5% HTS, or normal saline	No difference in 28-day mortality in HTS + dextran group (n = 231) compared to HTS group (n = 269) or saline group (n = 395) 25% vs. 27% vs. 25%, p = 0.91
ROC Hyporesus [38]	2015	Multicenter, randomized patients with hypotension (SBP <90) and GCS >8 to controlled resuscitation (250 mL boluses for SBP <70 or non-palpable radial pulse) or standard resuscitation (2000 mL bolus with additional crystalloid to keep SBP >110)	Lower unadjusted 24-hour mortality in controlled resuscitation group (n = 97) compared to standard resuscitation group (n = 95), but no difference in risk-adjusted mortality (p > 0.05) 5% vs. 15%, p = 0.03
PAMPer [39]	2018	Multicenter, cluster randomized helicopter bases (n = 27) to administration of two units of plasma or standard resuscitation in patients with severe hypotension (SBP <70) or hypotension and tachycardia (SBP 71–90 + HR ≥ 108)	10% reduction of 30-day mortality in plasma group (n = 230) compared to standard resuscitation group (n = 271) 23% vs. 33%, p = 0.03

(continued)

Table 29.1 (continued)

Trial	Published	Design and methods	Main result
COMBAT [40]	2018	Single center, randomized patients with severe hypotension (SBP <70) or hypotension and tachycardia (SBP 71–90 + HR ≥ 108) to receive two units of plasma or standard resuscitation with saline	No difference in 28-day mortality in plasma group ($n = 65$) compared to standard resuscitation group ($n = 60$) 15% vs. 10%, $p = 0.37$
RePHILL [41]	Recruiting	Multicenter, randomized patients with hypotension (SBP <90 or absent radial pulse) to receive prehospital blood product resuscitation (up to two units of PRBC and two units of freeze-dried plasma) or crystalloid resuscitation (up to four 250 mL normal saline boluses). Primary outcome is composite of in-hospital mortality and failure of lactate clearance ≥20% 2 hours after randomization. Enrollment goal of 490 patients	Pending
STAAMP [42]	Recruiting	Multicenter, blinded, randomized patients with SBP <90 or HR >110 within 2 hours of injury to receive 1gm bolus tranexamic acid or placebo. Primary outcome is 30-day mortality. Enrollment goal of 994 patients	Pending
PATCH [43]	Recruiting	Multicenter, blinded, randomized patients with prehospital Coagulopathy of Severe Trauma (COAST) score ≥3 within 3 hours of injury to receive 1gm bolus tranexamic acid or placebo. Primary outcome is 6-month mortality and Glasgow Outcome Scale-Extended. Enrollment goal of 1184 patients	Pending
TXA in TBI [44]	Completed	Multicenter, blinded, three-arm trial randomizing patients with GCS <13 to receive 1 gm tranexamic acid, or 2 gm tranexamic acid, or placebo. Primary outcome is 6-month Glasgow Outcome Scale-Extended. Enrolled 967 patients	Pending
FlinTIC [45]	Recruiting	Single-center, blinded, randomized patients with visible hemorrhage or clinical signs of bleeding to receive 50 mg/kg of fibrinogen concentrate or placebo. Primary outcome is fibrinogen polymerization. Enrollment goal of 60 patients	Pending
PPOWER [46]	Recruiting	Single-center, randomized patients with severe hypotension (SBP <70) or hypotension and tachycardia (SBP 71–90 + HR ≥ 108) to receive two units of whole blood or standard resuscitation. Primary outcome is 28-day mortality. Enrollment goal of 112 patients.	Pending
PREHO-PLYO [47]	Recruiting	Multicenter, randomized patients with severe hypotension (SBP <70) or Shock Index >1.1 to receive freeze-dried plasma or normal saline resuscitation. Primary outcome is INR change from prehospital to admission. Enrollment goal of 140 patients	Pending

SBP systolic blood pressure, *mL* milliliters, *GCS* Glasgow Coma Scale, *HTS* hypertonic saline, *TBI* traumatic brain injury, *HR* heart rate, *PRBC* packed red blood cells

citation fluid for the foreseeable future in the vast majority of ground emergency medical service systems, despite advances in prehospital transfusion and resuscitation.

There is some evidence that patients with hypotension in the field benefit from crystalloid administration. Hampton and colleagues demonstrated that a 16% reduction in the hazard of mor-

A L G r a w a n y

tality was independently associated with a median infusion of 700 mL of prehospital crystalloid among patients requiring early blood transfusion upon arrival to the trauma center [54]. Another retrospective review of severely injured blunt trauma patients compared high (>500 mL) versus low volume of prehospital crystalloid stratified by prehospital hypotension. Patients without hypotension have a nearly 2.5-fold increase in mortality if receiving >500 mL of prehospital crystalloid; however, there was no increase in mortality for hypotensive patients [48]. Further, the highest mortality among hypotensive patients was among those receiving no prehospital crystalloid. A recent secondary analysis of the Prehospital Air Medical Plasma (PAMPer) trial demonstrated similar findings, with the highest mortality among severely hypotensive patients (systolic blood pressure <70 mmHg) receiving no prehospital crystalloid but the lowest mortality among patients receiving 1–500 mL when crystalloid was the only available prehospital resuscitation fluid [55]. The Resuscitation Outcomes Consortium conducted a pilot study that randomized patients with hypotension in the field to receive a 2 l crystalloid bolus plus fluid to maintain a systolic blood pressure >110 mmHg or receive 250 mL boluses only when systolic blood pressure was <70 mmHg or non-palpable radial pulse (Table 29.1) [38]. The group found the controlled bolus strategy resulted in a lower volume of prehospital crystalloid (average 1 l compared to 2 l) with lower unadjusted 24-hour mortality, but not adjusted mortality. This effect was predominantly in blunt trauma patients.

The harmful effects of hypotension in the field on outcome in patients with TBI are well documented, with a doubling of mortality for even a single episode of prehospital hypotension [56]. One evaluation of lowest field systolic blood pressure demonstrated an inverse relationship between survival and systolic blood pressure between 40 and 120 mmHg, suggesting no specific threshold abates the mortality associated with secondary insult in TBI [57]. Current guidelines recommend fluid therapy in the prehospital setting to maintain a systolic blood pressure >90 mmHg to prevent secondary insult, despite no direct evidence that raising the blood pressure improves survival or functional outcome [11]. One recent study demonstrated that implementation of prehospital TBI management guidelines was associated with more crystalloid boluses given, less hypotension on arrival to the trauma center, and reduced mortality in severe TBI patients [53].

Given current evidence, when crystalloid is the only prehospital fluid available to prehospital providers, very limited (<500 cc) or no crystalloid should be provided to non-hypotensive patients. Severely hypotensive patients may still benefit from small amounts of crystalloid, with 250 mL boluses targeting a systolic blood pressure of 70–80 mmHg, palpable radial pulse, or normal mental status, especially in blunt trauma without TBI. Providers should aim for a total volume of 500 mL to a maximum of 1 l. Patients with penetrating torso trauma should receive limited or no prehospital fluid, and prehospital access/resuscitation attempts should not delay transport to a trauma center. Finally, in the absence of additional evidence, patients with suspected TBI should receive crystalloid boluses targeting a systolic blood pressure >90 mmHg.

Prehospital Blood Products

With mounting evidence of the deleterious effect of crystalloids in severely injured patients, the focus is now on damage control resuscitation with blood product component resuscitation and attention to the ratio of plasma and platelets to PRBC. The goal is to restore tissue oxygenation and a more physiologic coagulation milieu with the repletion of coagulation factors and platelets while avoiding the pro-inflammatory and dilutional coagulopathy induced from crystalloid infusion. This strategy has shifted toward earlier and higher ratio of blood product components, with a more balanced component transfusion in an attempt to approximate what is lost—whole blood. Given the success of damage control resuscitation employed early in the hospital setting [1, 2, 58, 59], it only makes sense to push

this strategy into the field to address hemorrhagic shock as early as possible. Data demonstrating death from hemorrhage occurs within the first 3 hours from injury and one-third of deaths from exsanguination occur in the field highlight the critical window for blood product administration in the prehospital setting [60, 61].

The initial experience with prehospital blood product resuscitation dates back to military medicine in World War II [62] (see Chap. 1). More recently demonstrated in Iraq and Afghanistan, prehospital blood product resuscitation has shown improved survival and has become the standard of combat casualty care when available [63–67]. Guidelines for logistics and safety of such practices in civilian prehospital trauma care have prevented widespread generalizability until lately. A survey of level 1 and 2 trauma centers participating in the Trauma Quality Improvement Program (TQIP) indicated that 34% of emergency medical services have the capability to administer prehospital blood products [7].

Packed Red Blood Cells

Packed red blood cell transfusion is the most commonly available prehospital blood product [7]. To date PRBC capabilities have generally been limited to air medical transport agencies, and early evidence has shown the practice to be both safe and feasible [68, 69]. Although prehospital PRBC transfusion has been available for decades in some areas, it is only recently that data have shown support for this practice.

The military evidence has shown improvements in mortality for patients receiving prehospital PRBC in recent conflicts. Deployment of advanced medical platforms with prehospital PRBC transfusion capabilities in the US and UK military resulted in greater than expected survival for severely injured patients [70]. Morrison et al. demonstrated that advanced prehospital capabilities including transfusion of PRBC in one-third of casualties demonstrated a 6% absolute mortality reduction among patients with injury severity score >15 [65].

The civilian evidence for the effectiveness of prehospital PRBC is mounting as well. Early studies evaluated small numbers of patients without the power to truly demonstrate effectiveness [71]. One small study of 50 propensity-matched patients receiving prehospital PRBC from the Glue Grant multicenter collaborative found a reduction in 24-hour and 30-day mortality, as well as lower risk of TIC as approximated by INR [72]. A larger single-center propensity-matched cohort of 240 air medical patients receiving prehospital PRBC after injury from the same group demonstrated that prehospital PRBC transfusion was associated with improved 24-hour mortality, lower risk of shock on arrival, and fewer PRBC required in the first 24 hours after admission [73]. A systematic review of prehospital PRBC evaluated 16 case series and 11 comparative studies [74]. The authors noted low quality of evidence with no overall effect on early or late mortality; however, they noted that studies which matched patients for severity of injury consistently suggested modest survival improvement.

Plasma

Use of prehospital plasma transfusion has gained increasing interest. Plasma has several advantages as a resuscitation fluid. Like PRBC, plasma is iso-osmolar with circulating blood and thus is an ideal fluid expander. Unlike PRBC, however, plasma contains the clotting proteins to directly address the TIC that occurs early in patients with tissue injury and hemorrhagic shock [40]. Finally, there is increasing evidence that endothelial glycocalyx degradation results in coagulopathy and endothelial dysfunction in hemorrhagic shock [75, 76]. In preclinical data, plasma has been shown to attenuate the disruption of the endothelial glycocalyx, improving outcome [77, 78].

As prehospital PRBC have long been available, few studies evaluate the sole effect of prehospital plasma resuscitation for trauma. The Mayo Clinic transport program added plasma transfusion capabilities in 2011 and reported the first five patients receiving plasma only with TBI

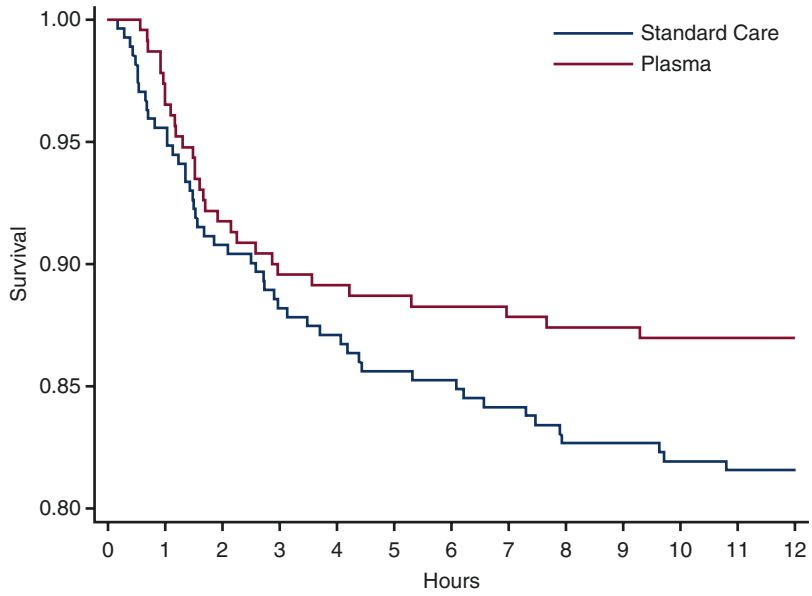


Fig. 29.2 Kaplan-Meier survival curve in the first 12 hours of patients receiving plasma or standard care resuscitation in the Prehospital Air Medical Plasma (PAMPer) trial

on warfarin for reversal [79]. All patients survived more than 24 hours and had a mean decrease of 1.2 in INR upon arrival at the trauma center. The same group updated their results in patients with TBI, comparing 36 patients receiving prehospital plasma to 40 patients receiving prehospital PRBC [80]. They found significantly improved neurologic outcomes at 6 months with higher functioning and lower disability among the prehospital plasma group.

With the promising results of preclinical and early clinical prehospital plasma data, the US Department of Defense issued a program announcement to evaluate prehospital plasma resuscitation for hemorrhagic shock in the civilian population. Ultimately two randomized trials were funded and completed, the PAMPer trial and the Control Of Major Bleeding After Trauma (COMBAT) trial (Table 29.1) [81, 82]. The multicenter PAMPer trial used a cluster randomized design by helicopter base to randomize air medical patients with severe hypotension or hypotension plus tachycardia to receive two units of thawed plasma or standard prehospital resuscitation with crystalloid or PRBC. A total of 501 patients were included and the plasma group had a 10% absolute reduction in 30-day mortality compared to the standard care arm [39]. The separation in the survival curve became evident beginning at 3 hours from injury (Fig. 29.2). There were lower 24-hour mortality, slight reduction in 24-hour transfusion requirements, and no difference in adverse events in the plasma group.

The single-center COMBAT trial randomized patients to receive thawed plasma or crystalloid in an urban ground emergency medical services system with plasma delivered upon arrival to the trauma center in both groups. A total of 125 patients were included and no difference in 24-hour or 28-day mortality was seen between the plasma or crystalloid groups [40]. When taking both trials into consideration, it becomes apparent that different populations were studied. The COMBAT trial was an urban population with median prehospital time of 26 minutes, while the PAMPer trial included air medical patients with a median prehospital time of 41 minutes. Thus, it seems early plasma transfusion is necessary, whether at the trauma center when prehospital times are short or in the prehospital setting with prolonged prehospital times.

Packed Red Blood Cells and Plasma

Following the paradigm of damage control resuscitation in the hospital setting, investigation of resuscitation with a balanced ratio of plasma and

PRBC in the prehospital setting is ongoing. Early results by Kim and colleagues in nine patients receiving prehospital PRBC and plasma compared to only PRBC suggested adding plasma resulted in greater improvement in coagulation status, higher plasma to PRBC ratio over the first 24 hours, and less crystalloid infusion [83]. As the military has added prehospital plasma capabilities to forward medical units, a retrospective matched cohort study of US combatants who experienced traumatic amputation or shock demonstrated that prehospital administration of PRBC and plasma resulted in a 15% and 12% reduction in mortality at 24 hours and 30 days, respectively [67]. Notably, of all injured patients who died, 70% where prior to hospital arrival, and of those, 74% were not transfused, again stressing the potential benefit of prehospital transfusion. A review of matched patients in the UK military experience also indicated an 11% reduction in casualties receiving prehospital PRBC and plasma transfusion [66].

Holcomb et al. reviewed their early experience of prehospital PRBC and plasma transfusion in their air medical transport program [84]. They compared 137 patients receiving PRBC and plasma to 169 controls with crystalloid only, demonstrating lower early hemorrhage rates and very early death from exsanguination in the first 6 hours, but no difference in 24-hour or 30-day mortality. A follow-up multicenter prospective study from this group compared air medical transport systems with PRBC and plasma to those without prehospital transfusion capabilities [85]. They did not find a difference in mortality; however, the data was hampered by significant differences in injury severity among patients receiving transfusion, as most systems now transfuse any severely injured patient with prehospital blood products when available. Most recently, Guyette and colleagues found that patients who received both PRBC and plasma in the PAMPer trial had the greatest survival benefit over patients receiving either PRBC or plasma alone (Fig. 29.3) [55]. A meta-analysis of prehospital transfusion suggested a pooled reduction in the odds of long-term mortality for prehospital transfusion of both PRBC and plasma, but not for PRBC alone [86].

Considering the body of evidence for improved outcomes with damage control resuscitation in the hospital setting and the more recent prehospital data, trauma patients at risk for hemorrhagic shock should be resuscitated with balanced blood product components as close to the time of injury as possible to prevent the development of coagu-

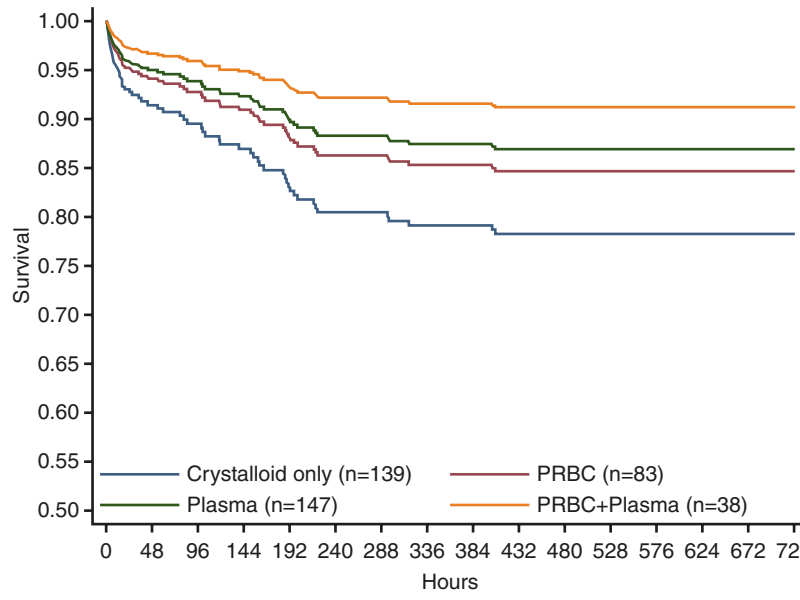


Fig. 29.3 Cox proportional hazards regression-adjusted survival curves of patients receiving crystalloid only, packed red blood cells, plasma, or packed red blood cells and plasma in the Prehospital Air Medical Plasma (PAMPer) trial

lopathy and the ensuing shock and inflammatory state associated with early mortality, most commonly within 3 hours. High ratio blood product replacement in essence reconstitutes whole blood, and current data shows the use of PRBC and plasma is feasible within modern emergency medical service transport programs [87]. The benefit of this approach is less clear in urban ground emergency medical systems with short transport times to a trauma center with damage control resuscitation capabilities.

Platelets

There is evidence that platelets are a critical component of damage control resuscitation. Evaluation of platelet transfusion has shown that higher early ratios of platelet to PRBC transfusion are associated with reduced mortality, and platelet transfusion in the PROPPR trial was associated with lower early mortality and improved hemostasis [88–90]. These data suggest early platelet transfusion in the field may be beneficial, despite limited availability of platelets in the prehospital setting [91]. At current, no studies evaluate outcomes of prehospital platelet transfusion, although the Mayo Clinical transport program recently added cold stored platelets to their prehospital transfusion capabilities [92]. Storage in the prehospital environment presents a particular challenge for platelets, but given the evidence for prehospital PRBC and plasma, cold stored platelets and whole blood storage that retains platelet function are receiving increasing interest [93–95].

Logistical Considerations

There are several challenges associated with a prehospital transfusion program. Foremost is a good working relationship with the blood bank that will be supplying products to the prehospital agency. Agencies must determine what type of products they will carry. Many agencies carry universal donor products (O negative PRBC, AB plasma); however, given the limited supply of these blood types, arguments have been made for use of low titer O positive blood and A low titer B plasma [96, 97].

There are generally two models for prehospital blood product programs. For prehospital agencies that are based at a participating hospital, blood products may be obtained "on demand" from the in-house blood banks. This model significantly decreases regulatory oversight and costs to the agency; however, it is only available to units stationed at the hospital and may prolong response time while obtaining the required blood products. When bases are located away from a participating blood bank site, base accommodations must be made to store blood products on site. Depending on local practices and regulations, it may be necessary to certify the prehospital agency bases as satellite blood banks. The agency must then purchase blood products from the blood bank at a cost of $100 to $400 per unit depending on type of blood component, blood type, and regional availability. The base must also purchase a blood refrigerator for storage (approximately $3500–$7500). Prehospital personnel then become responsible for proper storage and transport, recycling of units to prevent wastage, and documentation for the blood products (Fig. 29.4).



Fig. 29.4 Blood product storage cooler, transport cooler, and blood product tracking log

Prehospital crews must undergo training for the proper care and storage of blood products. Products generally need to be kept between 1 and 6 °C. Crews must check products on a daily basis to ensure proper function of the storage refrigerator to maintain necessary temperatures, monitor expiration date, make sure products are free from contamination and proper functioning of transport coolers for missions. These responsibilities must be outlined in protocols for crews, as well as protocols for maintenance for the storage refrigerator and documentation of storage conditions. Policies must also be developed that outline how the blood will be transported on the vehicle or aircraft during missions.

Additionally, protocols must be adopted for ordering of new blood product units when transfused on a mission, as well as when products approach their expiration date. Agencies must work with their blood bank to determine when and how the products will be recycled back to an appropriate hospital blood bank for use in the general pool to prevent wastage. PRBC have a maximum shelf life of 42 days, liquid plasma of 21 days, and fresh frozen plasma of 5 days, although some lead time is necessary to allow for recycling into the blood bank inventory and release for transfusion prior to expiration. An inventory and expiration tracking log are essential and may be electronic or paper based.

Step-by-step protocols must be developed for the process and documentation of blood transfusion in the prehospital environment. The protocol must consider the applicable scope of practice to ensure transfusion falls within the scope of practice for the prehospital providers. Indications for transfusion must be clearly delineated, as well as process for direct medical command, and can be adapted from published protocols [73]. The protocol must also address monitoring, treatment, and documentation of potential transfusion reactions.

Finally, a strong quality assurance program is necessary. This must incorporate monitoring and benchmarking of appropriate patient selection for transfusion, transfusion reactions, product usage and recycling, as well as wastage due to expiration or out of range temperature. Thus, prehospital blood transfusion programs can come with significant expense in both equipment and training. An analysis of the thawed plasma air medical program employed in the PAMPer trial demonstrated an annual cost of $25,000–$30,000 per helicopter base; however, most of the cost was due to courier costs to recycle plasma units with a short shelf life of only 5 days [98]. They suggest that liquid plasma with a longer shelf life and efficient recycling systems can mitigate a significant proportion of this cost. Up-front costs can be an investment of well over $10,000, with maintenance costs of several thousand dollars annually; however, evidence suggests real benefits to patients, and we believe the costs are well worth it when feasible to implement.

Prehospital Resuscitation Adjuncts

Tranexamic Acid

Several resuscitation adjuncts have emerged as part of hemostatic and damage control resuscitation principles and are now receiving interest in the prehospital arena. The adjunct that has garnered the most attention is prehospital use of tranexamic acid (TXA). Since the CRASH-2 trial was published demonstrating a reduction in mortality from exsanguination when TXA was administered within 3 hours of injury and the greatest benefit when given within 1 hour of injury [99], prehospital administration has become an attractive therapeutic option. Subsequent military and civilian data suggested a potential increased risk of venous thromboembolic events despite potential benefits, highlighting the need for appropriate patient selection [100, 101] (see Chap. 11).

Several prehospital systems have implemented prehospital TXA protocols in both ground and air transport systems, showing early feasibility of TXA administration in the field [102, 103]. Given the recent implementation of TXA in the prehospital environment, long-term outcomes are lacking with mixed early results. A Swiss study demonstrated reduced fibrinolysis in 24 patients receiving prehospital TXA, but no change in clinical outcomes compared to a propensity-matched

cohort [104]. Neeki et al. propensity-matched 362 patients receiving prehospital TXA to a historical cohort and found lower mortality among patients receiving TXA, although there was no adjustment for secular trend and the study population had a low overall mortality rate [105]. Boudreau and colleagues found no difference in mortality for prehospital versus emergency department TXA administration; however, only 116 patients were included during the study period [106].

One criticism of the adoption of prehospital TXA based on the CRASH-2 trial is generalizability, as CRASH-2 was conducted in resource-poor environments without the capacity for damage control resuscitation. Thus, it's not clear the same benefits will translate to more developed trauma and prehospital systems that have the capacity to provide prehospital transfusion and early damage control resuscitation. To that end, there are three current multicenter randomized trials underway that evaluate prehospital TXA in developed trauma systems, including the STAAMP trial, the PATCH trial, and the Prehospital Tranexamic Acid Use for Traumatic Brain Injury trial (Table 29.1) [107]. The highly anticipated results of these trials will elucidate the efficacy and dosing of TXA in the prehospital environment.

Fibrinogen

Fibrinogen concentrate is another proposed adjunct for early resuscitation. Fibrinogen levels are the first to become critically low and are associated with higher mortality in both civilian and combat casualties with TIC [108–110]. Fibrinogen concentrate is logistically appealing for the prehospital environment as it does not require thawing or crossmatching, and high doses can be rapidly administered over minutes. Early results suggest potential mortality improvements in severely injured patients with TIC that received fibrinogen. Stinger et al. showed that higher fibrinogen in the form of plasma, cryoprecipitate, whole blood, or platelets per unit of PRBC in massively transfused patients was associated with reduced mortality [110]. Administration of fibrinogen concentrate based on thrombelastography led to lower than predicted mortality in one study [111]. Finally, universal administration of 3 g of fibrinogen concentrate led to higher survival compared to no fibrinogen or administration only when plasma fibrinogen levels were low among severely injured patients [112]. Prehospital data on fibrinogen administration, however, is lacking. Two ongoing trials are evaluating the effects of fibrinogen concentrate in prehospital resuscitation algorithms and will help to shed light on the potential benefits of this adjunct (Table 29.1) [45, 113].

Prothrombin Complex Concentrate

The final resuscitation adjunct that is receiving attention is prothrombin complex concentrate (PCC), available in either 3 factor or 4 factor formulations. PCC has gained popularity owing to its rapid reversal of vitamin K antagonist anticoagulation, particularly in patients with TBI [114]. PCC again is attractive for prehospital use given its ease of storage and administration. Evidence suggest that 4 factor PCC may reverse coagulopathy faster, resulting in fewer transfusions than 3 factor formulations [115]. A recent propensity-matched study demonstrated reduced mortality associated with coadministration of PCC with plasma compared to plasma alone in patients with TIC in the absence of vitamin K antagonist use [116]. An ongoing trial is comparing addition of PCC to fibrinogen concentrate for in-hospital resuscitation of patients with TIC [117]. Prehospital data on PCC is limited to case reports and 1 small case series of 34 patients receiving PCC for pre-injury warfarin anticoagulation from a rural air medical transport service demonstrating reduced time to reversal of anticoagulation [118–120]. PCC appears to show promise in the prehospital environment for patients with known vitamin K antagonist anticoagulation, particularly in the setting of TBI; however, this requires further study given the lack of robust data and potential for thrombotic adverse events in TIC patients without pre-injury anticoagulation.

Future of Prehospital Resuscitation

Whole Blood

Given significant benefits of PRBC and plasma administered in the field to injured patients, the future of prehospital resuscitation lies with optimizing prehospital transfusion strategies. Currently, the logistical challenges of storage and space restrictions limit widespread applicability. Prehospital transfusion programs are largely confined to air medical transport programs and a very small number of well-resourced ground transport agencies. The promising results of prehospital transfusion of both PRBC and plasma over a single blood product suggest the use of prehospital whole blood may be the ideal approach to prehospital resuscitation [55, 86]. Whole blood has long been used in the military, demonstrating improved survival over component therapy in combat casualties [121]. Recently, the Army Rangers have developed an O low-titer whole blood program to provide whole blood transfusion at the point of wounding.

Cold stored whole blood transfusion for trauma has gained increasing interest given the benefits of damage control resuscitation which aims to reconstitute whole blood through high component ratios. Initial safety of cold stored whole blood has been demonstrated [95]. Several trauma centers across the United States have added whole blood capabilities to their initial resuscitation algorithm of injured patients [92, 97, 122]. Whole blood is not without issues, however, including reduced and dysfunctional platelets. Future challenges involve improving platelet sparing filter technology for whole blood preparation.

Use of whole blood in the prehospital arena reduces the space required to carry and store both PRBC and plasma for prehospital agencies. Further, since PRBC and plasma have different shelf lives, using a single product (i.e., whole blood) reduces the risk for wastage and burden on prehospital providers to track and appropriately return PRBC and plasma on differing schedules.

Whole blood has begun to make its way into the prehospital environment in select locations. The Norwegian air medical transport program deployed cold stored whole blood in 2015 [92]. The Norwegian service has long had a progressive prehospital transfusion program given the challenging geography and long distances over 370 miles between trauma centers in the county. In Texas, two emergency medical service agencies near Houston became the first prehospital ground agencies to carry whole blood, followed shortly by air and ground providers in San Antonio [97, 123]. The Mayo Clinic transport program which has long been a proponent of remote damage control resuscitation has added cold stored whole blood to their capabilities recently [92]. These early implementors are collecting ongoing data to evaluate outcomes; however, no prospective comparative or randomized data exists for prehospital whole blood administration in trauma. Thus, investigators at the University of Pittsburgh are conducting a randomized pragmatic trial to evaluate the efficacy of prehospital whole blood compared to standard prehospital resuscitation practice with crystalloid and PRBC (Table 29.1) [46].

Freeze-Dried Products

Another exciting frontier for prehospital resuscitation is the use of lyophilized or freeze-dried products. The process involves applying low temperature, low moisture, and low pressure environment or spray-drying by aerosolizing the product into a high temperature chamber to remove moisture [124]. This obviates the need for cold storage of blood products in the prehospital environment. It also extends the shelf life to the order of years. Reconstitution is rapid and simple in the field with comparable physiologic activity [125], making freeze-dried products the ideal solution for prehospital resuscitation.

Lyophilization of red blood cells has been hampered by damage to the cells without cryoprotectants such as glycerol; however, significant progress has been made using novel processes



Fig. 29.5 RePlas® freeze-dried plasma kit manufactured by Teleflex® Incorporated. (Photo credit: Teleflex® Incorporated United States Securities and Exchange Commission Filing Form 8-K, May 3rd, 2018; available at: https://teleflexincorporated.gcs-web.com/node/18871/html)

that allow for small volumes of freeze-dried red blood cells with acceptable functional rehydration of cells [126]. Technology exists to freeze-dry platelets as well; however, the limiting factor has been safety concerns. Animal studies of lyophilized platelet transfusion demonstrate short activity, excess thrombogenicity, and splenic accumulation that limits clinical applicability in current form [127].

Freeze-dried plasma for prehospital use has been the focus of recent attention, particularly given the survival benefit seen in the PAMPer trial [39]. Freeze-dried plasma was used in World War II but abandoned due to high rates of hepatitis C (see Chap. 1), but pathogen reduction technology has eliminated this concern. Commercially available freeze-dried plasma products already exist from manufactures in Germany, France, and South Africa [128]; however, freeze-dried plasma is not Food and Drug Administration (FDA) approved for use in the United States due to historical concerns of infectious contamination risks [124]. Prehospital freeze-dried plasma has been used by French military and civilian trauma teams, Norwegian air medical transport services, and Israeli Defense Forces with data supporting feasibility in the prehospital environment [129–131]. A recent in-hospital pilot trial of freeze-dried plasma compared to fresh frozen plasma suggested freeze-dried plasma achieved higher fibrinogen concentrations and better thrombelastography parameters [132]. Large-scale studies of outcomes for prehospital administration of freeze-dried plasma are awaited from two ongoing phase III trials (Table 29.1) [133].

Freeze-dried plasma has received particular interest from the US military. The FDA recently approved the use of freeze-dried plasma for US military while evaluating civilian approval of the product [134]. The US Army is supporting the development of US-based freeze-dried products (Fig. 29.5) [133], and US special forces are currently carrying the French manufactured product [128]. The US military is also planning a multicenter trial to evaluate outcomes of prehospital freeze-dried plasma administration in collaboration with civilian trauma systems.

Summary

The onset of physiologic derangements including the development of TIC occurs within minutes of injury. Prehospital resuscitation is increasingly recognized to have significant influence on injured patients' outcomes. Intravenous access with large-bore peripheral sites is the standard, although intraosseous access is gaining popularity when patients are difficult to obtain intravenous access or are in extremis. Crystalloid is the

mostly widely available prehospital resuscitation fluid but has pro-inflammatory effects and can exacerbate TIC. Prehospital crystalloid volume should be minimized if any is infused, although patients with severe hypotension or TBI may benefit from a moderate amount of crystalloid when it is the only resuscitation fluid available. Prehospital blood product transfusion has shown improved outcomes over crystalloid and is rapidly becoming the standard of care for well-resourced air medical transport programs to treat hemorrhagic shock. Plasma in particular has strong supporting evidence in patients with prolonged transport times. Resuscitation adjuncts including tranexamic acid, fibrinogen concentrate, and prothrombin complex concentration are easily administered in the prehospital environment and show promise; however, prehospital outcome data are lacking. Whole blood may be an ideal resuscitation fluid in the prehospital setting, allowing damage control resuscitation in the field while minimizing the number of products that need to be stored, carried, and administered by prehospital providers. Logistical challenges of storage are the primary barrier limiting widespread prehospital transfusion programs, and freeze-dried products may eliminate these barriers, making prehospital damage control resuscitation accessible to all injured patients with hemorrhagic shock in the future.

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Part VI

Management of Post Injury Hypercoagulability

Venous Thromboembolism After Trauma

30

Jonathan P. Meizoso and Kenneth G. Proctor

Introduction

On November 9, 1945, General George S. Patton, the brilliant field general and strategist of the European Theater in World War II, was involved in an automobile crash on his way to go pheasant hunting in Bavaria. He suffered a compression fracture and dislocation of the cervical third and fourth vertebrae, resulting in a cervical spinal cord injury that rendered him paralyzed from the neck down. He was kept immobilized and eventually placed in a total body plaster cast on November 20, 1945. He did well until the afternoon of November 21, 1945, when his condition dramatically worsened. In the words of his physician, Major General Albert W. Kenner:

> …he had a shower [of] emboli that hit his right chest and he started to fill up with his own sputum. We managed to get that pretty well under control, only to see him die very suddenly as a result of another shower of emboli. As a matter of fact, he went out like a light and certainly suffered no pain. The service lost its best field commander and I lost a damn good friend [1].

The association of PE with trauma was first reported by J.S. McCartney 11 years before Patton's untimely death [2]. McCartney emphasized the importance of immobilization as a contributing factor to fatal PE, demonstrated the increased frequency associated with fractures of the spine, and noted that most emboli occurred in the second week after trauma. Despite the passage of 80 years since McCartney's clarion call, VTE, including PE and DVT, remains a significant cause of morbidity and mortality following trauma.

Incidence of Venous Thromboembolism After Trauma

The "true" incidence of VTE after injury is a source of passionate debate, as is the discussion of whether asymptomatic or "silent" VTE events are clinically relevant. Several factors are integral in determining which patients develop VTE and whether we will detect them, including modifiable and non-modifiable risk factors, the use of chemical or mechanical thromboprophylaxis, and the use of screening and surveillance imaging modalities. It is now clear that symptomatic VTEs are only the "tip of the iceberg." Most cases of DVT are asymptomatic and most PE are

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clinically silent, making the true incidence of VTE incredibly difficult to define.

The prevalence of VTE risk factors (see Table 30.1), such as older age, spinal cord injury, and obesity (see section "Risk Stratification for Venous Thromboembolism After Trauma"), increases the reported rate. In a study stratifying trauma patients based on the presence of VTE risk factors, Bandle and colleagues found a DVT rate of 17% despite prophylaxis among the group at highest risk [3]. Patients in the groups at moderate risk and high risk had a combined DVT rate of 5%.

Thromboprophylaxis also affects the incidence of VTE following trauma. In a classic study by Geerts and colleagues, serial plethysmography and venography were used to prospectively evaluate a cohort of 349 trauma patients [median Injury Severity Score (ISS) of 26] for VTE [4]. None of the patients received any prophylaxis. Overall, 201 (58%) were diagnosed with DVT. Only three (1.5%) were symptomatic or had clinical features suggesting DVT. Proximal (above-knee) thrombosis occurred in 63 (18%) patients, while the rest were located below-the-knee in the tibial and calf veins. These results demonstrate that the vast majority of DVT are asymptomatic and that objective diagnostic imaging of asymptomatic patients (i.e., screening and surveillance) is essential to accurately assess the incidence of DVT. Reports of DVT only in patients who are symptomatic will grossly underestimate the magnitude of the disease. In a follow-up study by the same group, they showed that chemical thromboprophylaxis reduced thromboembolic events [5]. Patients were randomized to receive subcutaneous low-dose unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). Venography was again used to assess symptomatic and asymptomatic patients. Of the 265 patients included in the study, 100 (38%) developed DVT. Proximal DVT occurred in 11%. Subsequent studies using duplex ultrasound surveillance of the lower extremity have

Table 30.1 Risk factors for venous thromboembolism (Bandle et al. [3])

Highest-risk patient (any of these criteria)
Spinal cord injury with neurological deficit
Unstable spine fracture (requiring fixation)
History of DVT or PE
Hypercoagulable state
Factor V Leiden, protein C/S, or antithrombin deficiency, hyperhomocysteinemia, prothrombin mutations, anticardiolipin antibody
Four or more "high-risk" criteria
High-risk patient (any of these criteria)
ISS ≥10
Head AIS score ≥3
Pelvic fracture
Age >70 years (with additional "moderate-risk" criteria)
Mechanical ventilation for ≥3 days
Major venous injury/repair
Long-bone fracture of lower extremity
Presentation in shock (SBP on arrival of ≤80 mmHg or base deficit ≥5)
Insertion of femoral venous catheter
Central line placement
Active malignancy (not in remission)
Varicose veins
Recently postpartum (1 month) or current pregnancy
Extrication from MVA at scene
Myeloproliferative disorder
Sickle cell disease
Obesity (BMI ≥30)
Stable spine fracture
Operative time ≥2 hours
Transfusion of ≥4 units of PRBC
Moderate-risk patient
ISS ≥9
Age ≥70 years (without additional risk factors)
Age 40–70 years (with one additional "moderate-risk" factor)
Foot/ankle/fibula fracture
Inflammatory bowel disease (Crohn's disease or ulcerative colitis)
Additional "moderate-risk" factors
Supplemental estrogen use/hormone therapy
Prior diagnosis of cancer
History of myocardial infarction
Congestive heart failure
Chronic obstructive pulmonary disease
Current smoking

reported rates of DVT in the range of 2.5–9% in patients receiving a combination of mechanical and chemical prophylaxis [3, 6, 7].

The "true" incidence of DVT is also sensitive to surveillance bias. This occurs when variation in the use of screening and detection influences the frequency of an outcome or "the more we look, the more we find." [8] Pierce and colleagues documented evidence of surveillance bias in DVT reporting among hospitals in the National Trauma Data Bank (NTDB) [8]. The hospitals were separated into quartiles according to the use of venous duplex ultrasound, an objective imaging test to diagnose DVT in symptomatic and asymptomatic patients. The DVT rate observed in the highest quartile of ultrasound use was sevenfold higher than the average combined DVT rate for the other three quartiles. The potential magnitude of this surveillance bias was also demonstrated by a study from two level 1 trauma centers: Scripps Mercy Hospital, which used serial venous duplex ultrasound surveillance, and Christiana Care Health System, which used duplex ultrasound only for symptomatic patients [9]. Both centers closely followed accepted guidelines on mechanical and pharmacologic prophylaxis. Scripps Mercy treated 772 patients and Christiana Care treated 454 patients. The ISS and VTE risk were similar. The incidence of PE was 0.4% at both centers, but DVT was 5.3 times more likely at Scripps Mercy than at Christiana Care ($p < 0.0001$) [9]. Of the 80 patients who developed DVT, PE, or both, 99% received prophylaxis before the event. In those who received pharmacologic prophylaxis, the VTE rates between the two centers were not significantly different [9]. These results demonstrate that the odds of VTE are increased greater than five times when a simple surveillance program is used in trauma patients. Furthermore, neither pharmacologic nor mechanical prophylaxis was completely effective in preventing VTE in trauma patients, which supports the idea that VTE should not be considered a "never event" [10]. Thus, variability in the use of duplex ultrasound results in variability in the reported rates of DVT, further underscoring the impact of screening and surveillance in DVT case finding.

Compared with DVT, the incidence of PE reported in studies is relatively low, around 0.3% [11–14]. It is nonetheless a formidable problem with a mortality rate between 17% and 26% [11, 12, 15, 16]. Indeed, PE is a leading cause of death after the first 24 hours of admission following injury and is thought to be responsible for approximately 12% of all deaths due to trauma [17, 18].

Like DVT, surveillance bias affects the incidence of PE. With the advent of noninvasive diagnostic imaging, more specifically, contrast-enhanced thin-slice multidetector computed tomography (CT), more patients are being evaluated for PE. Furthermore, increasing numbers of incidentally discovered subclinical PEs are being diagnosed on CT scans performed for other reasons. As a result, the incidence of PE in trauma patients is increasing. Schultz and colleagues prospectively studied 90 consecutive patients with moderate-to-severe injury (ISS >9) who had no PE or DVT symptoms [19]. Utilizing multidetector helical CT scanning, the authors found a PE in 22 (24%) of 90 patients. Knudson and colleagues examined data from the NTDB to compare the incidence of PE reported between 1994 and 2001 (historical cohort) to that reported between 2007 and 2009 (current cohort) [14]. Patients in the historical cohort were younger and less severely injured than those in the current cohort. PE occurred in 0.21% of patients in the historical cohort and in 0.49% ($p < 0.01$) in the current cohort. Despite the significant increase in ISS and age, the mortality rate associated with PE decreased from 15% in the historical cohort to 11% in the current cohort. Only 20% of the patients with PE had an associated DVT. This could be explained, at least in part, by centers that only reported symptomatic DVT; thus, DVT associated with PE would be underreported because asymptomatic DVT was not detected. Finally, the idea that some PEs arise de novo as a result of local tissue injury and inflammation rather than as an "embolus" from a DVT must be considered (see section "Pathogenesis of PE" under section "Pathogenesis of DVT and PE," below). Indeed, the identification of different risk factors for DVT and PE, and the diagnosis of PE

in patients without evidence of DVT, has caused several investigators to question the teaching that PE is always a consequence of DVT [14, 20–22]. It is likely that primary pulmonary thrombosis is a distinct clinical entity from DVT.

It should be noted that the risk of VTE after injury does not disappear upon discharge from the hospital. In a population-based case-cohort study of 200 trauma patients in one Minnesota county, all residents with VTE after an acute hospitalization for trauma were reviewed over an 18-year period [23]. The median time from trauma to VTE was 18 days, with 62% of VTE identified after hospital discharge. VTE events were identified up to 3 months after discharge. Godat and colleagues reported similar results when reviewing the California Office of Statewide Health Planning and Development hospital discharge database [24]. The incidence of VTE was highest in the first 3 months after trauma at 10.3% and returned to that of the normal population (i.e., 0.1–0.2%) within 12–15 months. However, these are likely gross underestimates, given that 30% of VTEs are readmitted to a different hospital after the index admission [25].

In summary, the incidence of VTE reflects the aggressiveness of the pursuit of the diagnosis. Studies reporting the incidence of symptomatic DVT without screening or regular surveillance will underreport the true incidence. Studies that utilize screening and surveillance in combination with mechanical and pharmacologic prophylaxis will report an incidence of DVT between 2.5% and 17%, depending on the level of risk for VTE in the study population. Proximal DVT represents about 30% of DVT diagnosed in trauma patients, while below-knee DVT represents the remainder. The incidence of PE appears to be increasing, but the mortality associated with PE is decreasing. This increase may be due to surveillance bias owing to the recent use of more sensitive multidetector CT scanners. Symptomatic PE occurs in about 1–2% of *severely* injured trauma patients and is highly dependent on the clinical threshold for obtaining a diagnostic study. Finally, the risk of VTE after trauma remains elevated until at least 3 months from the time of injury and appears to normalize after 1 year.

Pathogenesis of DVT and PE

The etiology of thrombus formation in the venous system differs from that in the arterial system. Arterial thrombus contains erythrocytes, fibrin, and platelets. Venous thrombus, in addition to erythrocytes, fibrin, and platelets, also contains leukocytes—the presence of which plays an important role in the pathogenesis of VTE. Historically, the finding of leukocytes in the thrombus led many early physicians to suspect inflammation as a cause of thrombosis, particularly when they observed thrombus in proximity to an abscess [26].

Our basic understanding of the pathogenesis of venous thrombosis can largely be attributed to Rudolf Virchow, whose contributions to the field of vascular biology include the well-known "Virchow's triad," as well as the coining of the terms "thrombosis" and "embolus" [26]. Virchow's triad describes three elements—stasis, venous injury, and hypercoagulability—that are necessary for thrombus *propagation*, however, not for thrombus *generation*. In fact, historians Bagot and Arya credit Ludwig Aschoff by summarizing the elements that predispose thrombus *generation* [26]. These include:

- Changes in blood plasma (diminished or increased coagulability)
- Changes in blood elements (increased or diminished powers of agglutination)
- Changes in blood flow (slowing and formation of eddies)
- Changes in vessel wall itself (endothelial damage) [27]

The trauma patient represents the "perfect storm" of causative factors for thrombogenesis—the patient is injured, often immobilized, and hypercoagulable.

Venous Stasis

It should come as no surprise that stasis plays a major role in thrombus generation. Any disruption in the normal laminar flow of blood that results in eddy currents, turbulence, or blood stasis can bring platelets in close contact with the endothelium and create conditions favorable for clot formation and propagation. Early support for stasis as a risk factor for DVT was provided by Gibbs, who observed that DVT was strongly associated with the duration of bed rest in hospitalized patients and in the paralyzed limbs of patients suffering a stroke [28]. These observations were corroborated by the seminal autopsy series of 756 trauma/burn patients by Sevitt and Gallagher [29]. They found the highest frequency of thrombosis in the veins of the soleus and gastrocnemius muscles as well as the tibial veins of the leg. They also noted from the hospital records that DVT was associated with prolonged bed rest. At the time of their report, two views on the pathogenesis of DVT prevailed. One view was that the thrombus originates in the small soleus and gastrocnemius muscle veins, where flow velocity dramatically diminishes when the muscle(s) is not contracting. The thrombus then propagates proximally into the popliteal vein, where it either remains or embolizes to the pulmonary circuit. The other view was that venous thrombus arises de novo, even in larger veins with relatively high flow velocity, such as the iliac or femoral vein. Sevitt and Gallagher found both continuous thrombus on necropsy, extending from the soleal plexus all the way to the iliac vein, and discontinuous clot and isolated thrombi in injured and uninjured limbs [29], thus illustrating that both prevailing views on the pathogenesis of DVT had merit and, by extension, that stasis per se was not necessary to generate DVT. Nonetheless, small studies have shown that a structured progressive mobility protocol can improve circulation in critically ill trauma patients and reduce the incidence of VTE [30]. Similarly, a Cochrane review of five randomized controlled trials and three quasi-randomized trials using neuromuscular electrical stimulation systems (NMES) aimed at reducing venous stasis found that NMES was associated with lower risk of DVT compared to no prophylaxis in patients with spinal cord injury or in others in whom pharmacological or standard mechanical thromboprophylaxis is contraindicated, unsafe, or impractical [31]. However, the authors caution that the best available evidence for NMES is not robust enough to allow definitive conclusions about its use.

At the molecular level, Lopez and Chen posited that low venous flow or stagnation facilitates the local accumulation of procoagulants (e.g., thrombin), which typically are washed out by muscle contraction with leg movement [32]. Indeed, time to peak thrombin generation is an independent predictor of VTE after trauma [33]. In the absence of leg muscle contraction during bed rest or immobilization, procoagulants accumulate in valve pockets or the small sinusoids in the gastrocnemius and soleus muscles. Stasis also results in rapid desaturation of hemoglobin in local erythrocytes, resulting in activation of leukocytes, platelets, and endothelial cells. Activated endothelial cells release the content of Weibel-Palade bodies, which contain von Willebrand factor and membrane-bound P-selectin. Both of these proteins remain attached to the endothelial surface and bind leukocytes—a distinguishing pathologic constituent of the venous thrombus—that have the capacity to synthesize tissue factor, particularly during periods of hypoxia. Thrombus formation is then further enhanced by local platelet activation.

Venous Injury

Venous or sinusoidal wall injury can occur as a direct result of mechanical trauma, such as an adjacent fracture or muscle contusion. This exposes the subendothelial matrix, a rich source of tissue factor, which initiates the process of thrombin generation [34]. When injury is severe enough to occlude the vein or reduce flow, velocity conditions are sufficient for the initiation of DVT. Thrombus is also prevalent at vein junctions, where the vessel wall is relatively thin and more likely susceptible to injury and subsequent

exposure of the subendothelial matrix [32, 35]. However, endothelial injury adjacent to a fracture does not always produce DVT [29]. Thrombus can also occur in a vein segment more proximal to the injury, in a vein remote from the injury, or can also occur in an uninjured limb.

Thrombus remote from the site of injury was demonstrated by Schaub and colleagues, who investigated the early events that might contribute to postoperative DVT [36]. They performed transmission and scanning electron microscopy on the jugular and femoral veins of canines exposed to one of three types of surgery: splenectomy, hysterectomy, or intestinal division and reanastomosis. Each dog was followed for 4 hours while paralyzed and anesthetized. The venous endothelium was examined for injury and the adhesion of blood elements and debris to the luminal surface [36]. Responses were compared between the three groups and control animals that had no operation but remained anesthetized and paralyzed for 4 hours. Control veins (both femoral and jugular) showed a smooth endothelial carpet with intact cellular junctions that had only rare cells and minimal noncellular debris attached to the luminal surface. The endothelium of the veins from splenectomized animals had raised endothelial junctions with pseudopod formation and attachment of cells, mostly leukocytes and platelets and noncellular granular and particulate matter. The femoral veins from these animals also showed microthrombi. Endothelial changes and attachment of cells, granular material, and fibrinous debris were more frequent and more severe in the animals having either hysterectomy or intestinal anastomosis. In areas free of cells or debris, the endothelial surface was similar to that of the control animals. However, in areas where leukocytes and platelets were attached, endothelial alterations were also present. These included indistinct cellular junctions and pseudopod formation [36].

It appears from Schaub's work [36] that abdominal operative trauma can evoke a systemic effect by activating remote venous endothelium—perhaps changing its "phenotype" to a procoagulant one. If this is indeed the case, it provides a mechanism to explain the observations of Sevitt and Gallagher [29], namely, that DVT can occur in veins remote from the sites of injury or stasis. The attachment of leukocytes in the presence of remote endothelial alterations implicates a circulating chemotactic stimulus that activates apparently uninjured endothelium, not unlike that which occurs during inflammation. One may speculate that the attached leukocytes aggravate local endothelial cell injury and activation. The normal-appearing endothelium in the control (anesthetized and paralyzed) animals further suggests that relatively brief periods of stasis without trauma have little effect on remote veins.

Hypercoagulability

Trauma patients are generally hypercoagulable after injury, and several mechanisms have been proposed to explain this phenomenon. These include decreased function of antithrombotic substances (e.g., antithrombin III) and increased expression of prothrombotic elements (e.g., prothrombin, tissue factor). Others include resistance to activated protein C and a newly recognized pivotal role for platelets [37]. The potential critical role of fibrinolysis shutdown is reviewed in Chaps. 11 and 31. The increased incidence of VTE following tranexamic acid (TXA) administration has been associated with shutdown as discussed below.

Owings and colleagues demonstrated reduced antithrombin activity in severely injured trauma patients early in their hospital course, with antithrombin levels being lower in patients who developed DVT compared to those who did not [38]. Markedly elevated levels of prothrombin fragment 1 + 2 and d-dimer at hospital admission have also been described; however, elevation of these markers was not associated with an increased incidence of DVT [39]. Utter and colleagues found that, within hours of injury and for up to 3 days afterward, trauma patients express higher levels of monocyte-derived tissue factor [40].

Finally, viscoelastic testing has allowed us to confirm hypercoagulability following trauma more precisely than with usual plasma markers

[37, 41–43]. Harr and colleagues found that platelets contribute more to the hypercoagulable state than previously thought and advocated for the addition of antiplatelet agents to the current chemoprophylaxis regimens [37]. The impact of fibrinolysis shutdown on DVT, which is present at admission in 20% of seriously injured patients and may persist for at least 1 week postinjury in 50% of patients, is currently under investigation [44–46].

Pathogenesis of PE

The classical teaching regarding the etiology of PE is that they arise from a preexisting extremity DVT. However, the advent of noninvasive high-resolution CT has resulted in an increasing number of incidentally discovered PE, with fewer patients having an associated DVT [14, 20, 22], challenging the traditional paradigm that all pulmonary thrombi are true "emboli" from DVTs. Recent data support this paradigm shift. In a study examining the incidence of PE using the NTDB, Knudson and colleagues noted that some of the risk factors associated with PE in trauma patients differed from the risk factors for DVT, suggesting that not all PEs are embolic [14]. The authors found that patients with a severe chest injury had a 42% increased risk of PE with little likelihood of DVT. In contrast, patients with a major head injury were more at risk for DVT than for PE. Velmahos and colleagues found that only 15% of patients with PE had an associated DVT at their institution, again calling into question the relationship between PE and DVT [20]. Both Knudson and Velmahos hypothesized that some PE may arise de novo as a result of chest injury or local pulmonary inflammation. These are diagnosed incidentally on a chest CT done for reasons other than clinical suspicion of PE. They theorized that PE without DVT might constitute a different disease from PE associated with DVT. This theory is further supported by Van Gent and colleagues [22] who studied 2881 trauma patients at high risk for VTE who received duplex screening and surveillance of the lower extremities from ankle to groin. Thirty-one (1%) patients had a PE, but only 12 (39%) had an associated DVT. Patients with a PE, but no DVT, were younger, incurred more chest injuries ipsilateral to the PE, and had more peripheral clot. The authors surmised that local phenomena associated with injury or inflammation initiated the thrombus, thus coining the term "de novo pulmonary thrombosis." A follow-up study by the same group using competing risks analysis determined that risk factors for DVT and PE after injury were different [21]. DVT was associated with older age, severe injury, mechanical ventilation >4 days, active cancer, history of DVT or PE, major venous repair, male sex, and prophylactic enoxaparin and heparin use; PE was associated with younger age, non-severe injury, central line placement, and prophylactic heparin [21]. This further supports that DVT and PE are likely clinically distinct events that should be considered independently. Despite these findings, Allen and colleagues reported a retrospective review of 1282 trauma ICU admissions, 402 of which were deemed high risk for VTE [47]. Not surprisingly, the incidence of DVT was higher in patients screened with duplex ultrasonography (11.6%) compared to those who were not screened (2.1%). DVTs in the surveillance group were managed with systemic anticoagulation (43%) or with inferior vena cava (IVC) filters (57%). In the surveillance group, the PE rate was 1.9% compared to 7% in the non-surveillance group ($p = 0.014$), which led to the conclusion that surveillance and early management of DVT may decrease the incidence of PE [47]. These results may have been subject to surveillance bias, so they await confirmation with a prospective trial [47]. The ninth edition of Antithrombotic Therapy and Prevention of Thrombosis guidelines in nonorthopedic surgical patients by the American College of Chest Physicians currently recommends against the use of routine surveillance venous duplex ultrasonography for the prevention of DVT [48]. These recommendations are based on the fact that it is unclear whether identification and treatment of asymptomatic DVT reduces the risk of PE or fatal PE.

Based on the foregoing, it appears that PE following trauma can arise from a disease entity dis-

tinct from PE associated with DVT. Indeed, the developing consensus is that PE may represent a composite of true emboli from peripheral DVT (representing 20–40% of cases) and de novo pulmonary vascular thrombosis (representing 60–80% of cases) associated with local injury or infection.

Risk Stratification for Venous Thromboembolism After Trauma

Patients with traumatic injury have the highest risk for VTE among all hospitalized patients [49]. In the absence of thromboprophylaxis, the incidence of DVT and PE approach 50% and 1%, respectively. However, the risk of developing VTE after trauma is not equivalent among all injured patients. Several risk factors have been described in the literature that confer an increased risk for postinjury VTE [3, 4, 17, 50].

One of the earliest studies aimed at investigating VTE after trauma was completed by Sevitt and Gallagher in 1961 [29]. In this autopsy study of injured patients, they found that the major inciting factors for DVT included age, immobility, and bed rest—all factors that remain just as significant in modern-day practice [29]. Shackford and colleagues developed a list of risk factors thought to increase the risk of VTE after trauma based on prior literature [50]. This list included age >45 years with mandatory bed rest >3 days, history of VTE, spine fracture without neurological deficits, coma with Glasgow Coma Scale (GCS) score <7, quadriplegia/paraplegia, pelvic fracture, lower extremity fracture, repair of a major lower extremity venous injury, and complex lower extremity wounds. In a cohort of 719 trauma patients who had serial surveillance screening, none of the patients without risk factors developed VTE compared to 7% of the patients with at least 1 risk factor. On logistic regression, they found that age >45 was the only risk factor independently associated with VTE [51]. Geerts et al. found an overall DVT incidence of 58% in 349 trauma patients admitted to a single level I trauma center who underwent serial surveillance exams [4]. Multivariate analysis revealed age [odds ratio (OR), 1.05; 95% confidence interval (CI), 1.03–1.06], blood transfusion (OR, 1.74; 95% CI, 1.03–2.93), surgery (OR, 2.30; 95% CI, 1.08–4.89), fracture of the femur or tibia (OR, 4.82; 95% CI, 2.79–8.33), and spinal cord injury (OR, 8.59; 2.92–25.28) as independent risk factors for VTE [4]. Of note, none of the patients in this study received thromboprophylaxis. Knudson and colleagues analyzed 1602 VTE episodes from the NTDB and identified age ≥40 years (OR, 2.01; 95% CI, 1.74–2.32), lower extremity fracture with Abbreviated Injury Scale (AIS) ≥3 (OR, 1.92; 95% CI, 1.64–2.26), head injury with AIS ≥3 (OR, 1.24; 95% CI, 1.05–1.46), ventilator days >3 (OR, 8.08; 95% CI, 6.86–9.52), venous injury (OR, 3.56; 95% CI, 2.22–5.72), and major operative procedures (OR, 1.53; 95% CI, 1.30–1.80) as independent risk factors for VTE after trauma [15]. The strength of these associations is limited, however, because the NTDB dataset lacks information on screening, surveillance, and prophylaxis. Finally, Meizoso and colleagues identified five risk factors strongly predictive of VTE after trauma in a large cohort of 1233 admissions to a single trauma center [51]. In multivariate analysis, these risk factors included ≥4 transfusions in the first 24 hours of admission (OR, 2.60; 95% CI, 1.64–4.13), GCS <8 for >4 hours (OR, 2.13; 95% CI, 1.28–3.54), pelvic fracture (OR, 2.26; 95% CI, 1.44–3.57), age 40–59 years (OR, 1.70; 95% CI, 1.10–2.63), and >2 hour operation (OR, 1.80; 95% CI, 1.14–2.85) [52].

Recent advances in our understanding of the coagulopathy of trauma have identified other pathophysiologic changes that confer an increased risk of VTE. Brill and colleagues found that a hypercoagulable TEG, defined as reaction time below, alpha angle above, or maximum amplitude above reference ranges, were associated with increased risk of DVT; the rate of DVT was twice as high in patients with hypercoagulable TEG despite appropriate thromboprophylaxis [53]. Others have noted similar findings [41, 54, 55].

A large, randomized, multicenter trial reported that the administration of the antifibrinolytic agent, TXA, within 3 hours of injury decreases

all-cause mortality [55–57]. Although the initial trial did not identify an increased incidence of thrombotic events in patients receiving TXA, other more recent papers have identified an association between TXA use and VTE after trauma [56–58]. While some argue that this is related to the induced state of impaired fibrinolysis produced by the drug (see Chaps. 26 and 31), others have not observed an association between TXA and VTE [56, 59, 60]. Other factors known to dysregulate the fibrinolytic cascade, such as the administration of fresh frozen plasma, have also been associated with VTE after trauma [61].

Two major risk assessment tools exist to stratify patients based on their likelihood of developing VTE after trauma. Greenfield's risk assessment profile (RAP), developed in 1997, identified 17 risk factors thought to increase the risk of VTE in trauma patients [62] (see Table 30.2). The sample size was small, with a total of 53 patients completing the study and an overall DVT incidence of 43%. "High-risk" patients were defined as those with a RAP score ≥5. This cutoff successfully identified a group of patients with at least three times greater risk of developing VTE than the average trauma population [62]. Interestingly, there was still a proportion of patients with RAP <5 who developed DVT. Despite widespread use, there is still some controversy regarding the utility of the RAP score. While some have found it a helpful adjunct, others failed to validate a RAP ≥5 as a sufficient cutoff for VTE risk stratification [63, 64]. Similar predictive ability has also been found using a simplified risk score incorporating only 5 of the original 17 risk factors in the Greenfield score [52]. The newer Thromboembolic Scoring System (TESS), developed by Rogers et al. in 2012, was derived using multivariate analysis in a cohort of 16,608 trauma patients and includes 5 variables: age, ISS, obesity, ventilator use for more than 3 days, and lower extremity trauma [65]. It was then validated using the NTDB dataset from 2002 to 2006 to determine its ability to predict VTE. TESS scores range from 0 to 14. The sensitivity and specificity for predicting VTE was highest with TESS >6 (81.6% and 84%, respectively; Youden index, 0.66) [65]. The area under the receiver operating characteristic curve derived for TESS using the NTDB dataset was 0.84 (95% CI, 0.83–0.84), indicating excellent predictive value of the model for VTE [65]. Zander and colleagues recently compared the two models to assess their validity in trauma patients for both symptomatic and asymptomatic VTE; both groups had received VTE prophylaxis [66]. Results revealed TESS had a sensitivity of 49% and a specificity of 72%, while RAP had a sensitivity of 83% and a specificity of 37%. Each of the two models was limited in accuracy, which might be remedied by incorporating additional, yet unidentified, risk factors.

In summary, each trauma patient presents with a unique constellation of demographic characteristics, injuries, and risk factors that interact to produce an outcome, which may well include VTE. Clinical judgment based on the calculus of harm is required, balancing the risks and benefits with regard to symptoms, use of screening and surveillance, and implementation of prophylactic measures.

Table 30.2 Greenfield's Risk Assessment Profile [62]

	Weight
Underlying conditions	
Obesity (>120% Metropolitan Life Table)	2
Malignancy	2
Abnormal coagulation factors at admission	2
History of thromboembolism	3
Iatrogenic factors	
Central femoral line >24 hours	2
Four or more transfusions during first 24 hours	2
Surgical procedures >2 hours	2
Repair or ligation of major vascular injury	3
Injury-related factors	
AIS >2 for the chest	2
AIS >2 for the abdomen	2
Spinal fractures	2
AIS >2 for the head	3
Coma (GCS <8 for >4 hours)	3
Complex lower extremity fracture	4
Pelvic fracture	4
Spinal cord injury with para or quadriplegia	4
Age	
>40 but <60	2
>60 but <75	3
>75	4

Thromboprophylaxis Strategies

All trauma patients should receive some form of prophylaxis against VTE. Several methods exist to prevent VTE after trauma. Chemical thromboprophylaxis consists of treatment with either unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). Mechanical thromboprophylaxis is most often achieved with intermittent pneumatic compression (IPC) devices. A recent systematic review and meta-analysis of thromboprophylaxis strategies in trauma patients found a significantly decreased risk of DVT in patients receiving any form of thromboprophylaxis compared to no prophylaxis [relative risk (RR), 0.52; 95% CI, 0.32–0.84] [67]. The optimal method of prevention is likely combined mechanical and chemical thromboprophylaxis, which was also noted to reduce the risk of DVT relative to chemical prophylaxis alone (RR, 0.34; 95% CI, 0.19–0.60) [67]. Thromboprophylaxis should be initiated as early as safely possible, as the risk of VTE increases if treatment is delayed beyond 72 hours [68]. Furthermore, treatment should not be interrupted as missed doses of thromboprophylaxis have been correlated with DVT formation [69].

Chemical Thromboprophylaxis

There are two major pharmacologic agents used for VTE thromboprophylaxis after trauma: UFH and LMWH. Strategies for VTE prophylaxis after trauma were adapted from elective surgical and general medical patients. UFH dosed at 5000 units (U) every 8 hours postoperatively is effective in reducing the incidence of VTE in patients undergoing elective surgery [70–72]. This reduction in VTE events is thought to be secondary to the potentiation of antithrombin III's inhibitory effect on thrombin, as well as factors Xa, IXa, XIa, XIIa, and TF-VIIa (See Chap. 4) by heparin [50]. LMWH is frequently dosed either at 40 mg subcutaneously once daily or 30 mg subcutaneously twice daily [73]. The mechanism of action for LMWH is similar to that of UFH, although its ability to directly inhibit thrombin is lower [74]. LMWH is believed to be advantageous due to the uniform molecular size compared to unfractionated heparin which consists of multiple molecular forms and, thus, less predictable biologic activity.

The success of UFH and LMWH as thromboprophylaxis agents in elective surgical patients led to their adoption in trauma. In the United States, LMWH has become the agent of choice for thromboprophylaxis after trauma. Several studies have demonstrated that LMWH may be superior to UFH for the prevention of DVT after trauma [5, 67, 75–80], although the mechanism is not well understood. Geerts and colleagues compared LMWH (30 mg subcutaneously twice daily) with UFH (5000 U twice daily) in a prospective randomized trial involving 344 patients, all of whom had significant injury with ISS >9 [5]. Patients underwent bilateral lower extremity contrast venography between 10 and 14 days after admission or just before discharge if discharged earlier. Of the 265 patients with venograms adequate for analysis, VTE incidence was 31% ($n = 40$) in the LMWH group compared to 44% ($n = 60$) in the UFH group ($p = 0.014$). No mechanical prophylaxis was used. In a randomized noninferiority trial comparing UFH dosed three times daily to enoxaparin for prevention of VTE after trauma, UFH was noninferior to enoxaparin [81]. The dosing strategy for UFH in this study may explain the different outcomes when compared to other studies. More recently, a review of 18,010 trauma patients in the Michigan Trauma Quality Improvement Program with ISS ≥5, and length of stay >24 hours, found a decreased risk of mortality, VTE, PE, and DVT, with LMWH compared to UFH [76]. Barrera and colleagues found that LMWH reduced the risk of DVT compared to UFH in a systematic review and meta-analysis of four randomized controlled trials in trauma patients (RR, 0.68; 95% CI, 0.50–0.94) [67]. Thus, the American College of Chest Physicians guidelines for the management of VTE [82], the level III recommendations from the Eastern Association for the Surgery of Trauma [50], and the majority of evidence support the use of LMWH, rather than UFH, for VTE thromboprophylaxis after trauma.

However, there is no consensus regarding optimal dosing strategies for LMWH. The use of antifactor Xa levels for monitoring and titrating enoxaparin doses has been an area of active investigation. While the standard dosing of enoxaparin 30 mg twice daily may be insufficient [83], there is uncertainty as to whether titrating enoxaparin based on antifactor Xa levels is beneficial. In a study of 205 trauma patients, 87 underwent antifactor Xa-based adjustment, and a historical control of 118 patients received enoxaparin 30 mg twice daily [84]. The incidence of VTE was significantly lower in the adjustment group compared to the control group (1.1% vs. 7.6%, $p = 0.046$). Dhillon and colleagues had similar findings in a cohort of 159 patients with lower extremity and/or pelvic fractures—VTE rates were 13.9% in the control group vs. 1.7% in the antifactor Xa-adjusted group ($p = 0.03$) [85]. Others have failed to find a therapeutic benefit to antifactor Xa-based dosing. Karcutskie and colleagues followed 194 trauma patients in the intensive care unit with a median ISS of 23 who were dose-adjusted based on antifactor Xa levels. Only 53% of patients ever reached the therapeutic antifactor Xa range, and no differences in VTE, DVT, or PE rates were noted between those who became prophylactic and those who did not [86]. In a follow-up study, they propensity score-matched 132 patients receiving standard fixed-dose enoxaparin to 84 patients receiving dose-adjusted enoxaparin and found no significant differences in the incidence of VTE between groups (2% vs. 4%, $p = 0.57$) [87]. Other dosing strategies for LMWH thromboprophylaxis include weight-based dosing prior to titration based on antifactor Xa levels. Most antifactor Xa-level based protocols start patients on a weight-based dose of enoxaparin and measure antifactor Xa levels. Enoxaparin doses are then adjusted to achieve antifactor Xa levels in the prophylactic range. Most studies show that this is an effective method to achieve prophylactic range antifactor Xa levels [88–90], although the effect on the incidence of VTE is controversial [91, 92]. Finally, TEG-based dosing of enoxaparin was not shown to decrease the incidence of VTE when compared to standard dosing (6.7% vs. 6.3%, $p > 0.99$) in a randomized, multicenter trial of surgical and trauma patients [93].

The major risks associated with thromboprophylaxis include bleeding and heparin-induced thrombocytopenia (HIT) [74]. Bleeding has been reported to occur in up to 2% of trauma patients receiving either agent [17]. HIT occurs even less frequently and the risk may be slightly higher with UFH than LMWH. One meta-analysis in surgical and medical patients receiving either LMWH or UFH found that the absolute risk for HIT was 0.2% for LMWH and 2.6% for UFH [94].

Certain special situations warrant further discussion. Renal function should be assessed prior to the administration of LMWH as this drug is renally cleared and should not be administered to patients with renal insufficiency (creatinine clearance <30 mL/minute) [95, 96]. The use of either LMWH or UFH in patients with traumatic brain injury is also considered contraindicated by some, although its use in patients with invasive intracranial monitors is not associated with increased bleeding complications [97]. Current evidence suggests that thromboprophylaxis is safe when initiated within 72 hours of injury in patients with TBI and a stable imaging and may decrease the incidence of VTE by 50% without increasing morbidity [98]. The American College of Surgeons Trauma Quality Improvement Program Best Practices Guidelines for the Management of Traumatic Brain Injury supports the initiation of thromboprophylaxis within 72 hours of injury in patients with TBI and a stable head CT [99]. The Brain Trauma Foundation guidelines also support the use of LMWH and UFH in TBI; however, they do not mention the optimal timing [100]. Similarly, early VTE thromboprophylaxis (within 48 hours) has been found to be safe and beneficial in patients with pelvic fracture [101], spinal cord injury [95, 97, 102–105], and blunt solid organ injury managed nonoperatively [97, 106–113].

As our understanding of the pathophysiology of VTE continues to evolve, new targets for prophylaxis will inevitably emerge. Recent findings have shown the integral contribution of platelets to the postinjury hypercoagulable state [37].

These findings have led to recommendations to add aspirin to the thromboprophylaxis regimen after trauma. Thromboprophylaxis with aspirin is not a new idea and has been used in orthopedic patients for decades [114–117]. However, its use in trauma patients requires further study (see Chap. 7).

IVC Filters

IVC filters have been recommended as thromboprophylaxis after trauma for patients with contraindications to chemical thromboprophylaxis [118–120]. The use of IVC filters for this indication has increased significantly [121]. A recent meta-analysis by Haut and colleagues found a consistent reduction of PE and fatal PE in trauma patients with IVC filter placement, with no difference in mortality from DVT [122]. However, the strength of the evidence was low, and the use of IVC filters for PE prophylaxis remains controversial [123]. Furthermore, studies showing no clear benefit from IVC filter placement and low retrieval rates associated with an increased risk of morbidity underscore the need for further study on their utility [124–126].

Mechanical Thromboprophylaxis

Intermittent pneumatic compression (IPC) devices contain an inflatable bladder that intermittently expands and compresses the gastrocnemius and soleus muscles to achieve an increase in venous flow velocity, thereby reducing stasis [127, 128]. The effects of IPC may not be solely mechanical [127]. These devices may also increase fibrinolysis, given transient increases in tissue plasminogen activator levels; however, this effect appears to decay within minutes of their discontinuation [129, 130].

Several studies have shown that IPC alone is more efficacious than no prophylaxis for reducing VTE after trauma [67, 131–134] and that IPC alone is effective as VTE prophylaxis in nontrauma hospitalized patients [135–137]. In contrast, a meta-analysis by Velmahos and colleagues found that IPC was equivalent to no DVT prophylaxis [138]. A more recent meta-analysis of five randomized controlled trials found that mechanical prophylaxis with IPC reduced the risk of DVT relative to no prophylaxis (4.5% vs. 8.8%, RR 0.43; 95% CI, 0.25–0.73) [67].

There are minimal risks associated with the use of IPC, except cost. Although skin necrosis and peroneal palsy have been reported [139, 140], these complications are exceedingly rare and may be related to improper application. IPC devices should be used continuously until ambulation [95]. However, there is a high rate of noncompliance, particularly in patients on general hospital units [141], which may limit their efficacy [141, 142]. The utility of IPC is further limited in patients with lower extremity fracture, external fixators, plaster immobilizers, or significant open wounds, which can occur in up to 35% of patients [51]. In these situations, foot IPC can be used [50, 143, 144].

Taken together, IPC devices are an effective option for thromboprophylaxis in trauma patients and are associated with minimal risk. IPC devices should be used routinely in patients who can tolerate them, regardless of whether or not the patient is receiving chemical thromboprophylaxis, given the decreased incidence of DVT with a combined approach [67].

In summary, most injured patients have at least one risk factor for the development of VTE and require some form of thromboprophylaxis. Chemical options include UFH and LMWH. LMWH is the preferred agent of choice in patients without contraindications. Several dosing strategies exist for LMWH, including standard dosing (30 mg subcutaneous twice daily), antifactor Xa-based dosing, and weight-based dosing followed by adjustments based on antifactor Xa. TEG-based dosing does not appear to confer any benefit in reducing VTE events. IPC should be used when possible. Careful consideration to using thromboprophylaxis is necessary in select patient populations, although its use appears to be safe in most subgroups within 48–72 hours after injury. IVC filter use in patients with contraindications to chemical thromboprophylaxis remains controversial. Despite all these

available strategies, VTE events continue to occur [10, 145], underscoring the need to investigate new strategies as we learn more about the pathophysiology of thromboembolic disease.

Treatment of VTE

VTE should be treated with therapeutic anticoagulation once diagnosed unless major contraindications exist. VTE recurrence can occur in up to 30% of patients within 10 years in the general medical population [146–148]. Initial management includes a heparinoid (UFH or LMWH) bridge followed by a transition to warfarin or other oral anticoagulant. The heparin bridge is required to avoid the risk of a paradoxical hypercoagulable state secondary to the depletion of the regulatory anticoagulant proteins C and S [146]. In the setting of a contraindication to therapeutic anticoagulation, such as high risk of bleeding or recurrent VTE despite adequate doses of anticoagulation, an IVC filter should be considered.

Several anticoagulant strategies exist for the treatment of VTE. In a recent systematic review and meta-analysis, Castellucci and colleagues demonstrated that LMWH plus warfarin was associated with a slightly lower rate of VTE recurrence compared to UFH plus warfarin [149]. The authors also showed that rivaroxaban and apixaban were associated with a lower risk of bleeding. However, it should be noted that most of the patients in the studies reviewed were medical patients rather than trauma patients. Nevertheless, these non-vitamin K antagonist (non-VKA) oral anticoagulants (i.e., apixaban, dabigatran, edoxaban, and rivoraxaban) may be better options for extended therapy because their dosages are fixed and they do not require routine monitoring of coagulation function [148]. Aspirin has also been given for long-term management of VTE; however, the recent EINSTEIN CHOICE trial showed that once-daily rivaroxaban reduced the risk of recurrent VTE by 70% compared with aspirin and did not significantly increase bleeding risk, making rivaroxaban a more attractive option [148]. Recent meta-analyses have also compared the risk of bleeding between the non-VKA oral anticoagulants and found that apixaban has the most favorable bleeding risk compared to aspirin, dabigatran, edoxaban, rivaroxaban, and warfarin [150]. Therefore, oral anticoagulation may be better tolerated when given in the form of a non-VKA agent relative to warfarin, with apixaban having the most favorable adverse event profile.

There is no consensus on whether the duration of anticoagulation therapy should exceed 3 months. As with thromboprophylaxis, the duration of therapy must be considered in the context of the individual patient. If trauma is the only risk factor in a young and otherwise healthy patient, 3 months of oral anticoagulation should be considered adequate. If other factors exist (e.g., prolonged inactivity or ongoing infection), 6 months is reasonable.

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Fibrinolysis Shutdown and Venous Thromboembolism

31

Gregory R. Stettler, Ernest E. Moore, and Hunter B. Moore

Introduction

Fibrinolysis is a normal physiologic process that is essential to maintain microvascular patency by removing extraneous fibrin clot [1]. The extremes of fibrinolysis, termed hyperfibrinolysis (excessive breakdown of clot) and fibrinolysis shutdown (resistance to clot breakdown), are associated with increased mortality [1, 2]. The mechanisms and clinical implications of these pathologic extremes and their temporal trends have been detailed in the previous chapter on fibrinolytic derangements following trauma (Chap. 11). While pharmacologic therapies, such as tranexamic acid (TXA), to attenuate hyperfibrinolysis have been studied extensively in trauma, studies to identify effective strategies to treat fibrinolysis shutdown are limited.

Low fibrinolytic activity can be defined by viscoelastic hemostatic assessment (VHA) [1]. The two commercially available VHA are thrombelastography (TEG) and rotational thromboelastometry (ROTEM). The exact viscoelastic measurement used to define fibrinolysis phenotypes varies depending on which viscoelastic assay (TEG vs. ROTEM) and, further, which type of TEG or ROTEM assay is used (activated vs. native) [3–5]. A recent study evaluating TEG and ROTEM showed that these assays have moderate agreement in identifying fibrinolysis phenotypes [6] in which the outputs from either assay can be used to identify low fibrinolytic states following severe injury.

Low fibrinolytic activity is defined as a LY30 of <0.9% by rapid TEG which has been associated with an increased mortality following injury, with a mortality of 17–22% compared to a physiologic level of fibrinolysis of 3–14% [1, 2]. The cause of death in the shutdown cohort was typically late death and more commonly associated with multiorgan failure[1, 2]. It has been a hypothesis that multiple organ failure is caused by microthrombotic complications stemming from the decreased clot breakdown associated with fibrinolysis shutdown [1, 2]. Acute fibrinolysis shutdown has also been recognized in septic shock as a factor associated with increased morbidity and mortality [7]. An association between the inflammatory response and fibrinolytic capacity has been identified in septic patients and is suggested as a mechanism for the morbidity associated with markers of elevated inflammation such as C-reactive protein [8]. Additional studies

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have shown a relationship between fibrinolysis shutdown and increased morbidity in transplant patients [9]. Further refinement on the definition of low fibrinolysis has recently been described in trauma patients as those with hypofibrinolysis (lack of fibrinolysis activation) and fibrinolysis shutdown (prior activation of fibrinolysis and subsequent inhibition of the system) [10]. There has been confusion in the literature, as both hypofibrinolysis and fibrinolysis shutdown have been used interchangeably to describe the same patient population. For simplicity, in this chapter we will refer to trauma patients as being in fibrinolysis shutdown, but future research is needed to better delineate hypofibrinolysis from fibrinolysis shutdown, as both manifest as low fibrinolytic activity on functional assays.

Fundamentals of Fibrinolysis Shutdown

Because clot formation and degradation are ongoing and tissue-specific under normal physiologic conditions, the molecular mechanisms regulating the fibrinolytic system are diverse and complex. The driving forces of both hyperfibrinolysis and hypofibrinolysis are discussed in detail in Chap.11, but key points on low fibrinolytic activity will be highlighted in this section. Plasminogen conversion to plasmin by tissue plasminogen activator (t-PA) and urokinase (uPA) is the dominant step in activation of fibrinolysis [11]. A lack of activators, plasminogen activator inhibitors, plasmin inhibitors, or structural changes to the clot can produce a low fibrinolytic state. Primary regulation of the fibrinolytic system is via serpins, a family of serine protease inhibitors that irreversibly bind to and complex the active site serine on plasmin [11, 12]. Examples of the recognized levels of regulation include (Figs. 31.1 and 31.2):

A. Tissue plasminogen activator is stored in the Weibel-Palade bodies in the endothelium [13]. The release of t-PA from the endothelium is multifactorial and related to increasing intracellular calcium levels from several surface receptors that bind thrombin, epinephrine, and histamine [14]. Regulation of this process is poorly understood, but a tendency for thrombotic complications has been appreciated in patients who have deficient t-PA release [15].
B. Plasminogen activators are inhibited by plasminogen activator inhibitors (PAIs). While PAI-1 can be found in the endothelium, macrophages, and even adipocytes, substantial PAI-1 is stored in platelets, accounting for 90% of circulating plasma PAI-1 [16–19]. Experimental data shows that PAI-1 is rapidly released from alpha granules after thrombin stimulation in its active form and complexes t-PA [17]. Furthermore, thrombin stimulation of hepatic endothelium increases PAI-1 release [20]. PAI-2 is also a member of this serpin family but only found in significant plasma levels during pregnancy [11].
C. Plasmin is regulated by the most abundant and fast-acting serpin, alpha 2-antiplasmin (a2AP), which is also contained in platelet alpha granules. The normal circulating level of a2AP is 70 ug/mL [21]. Plasmin that is released into circulation is immediately neutralized by this serpin [11]. This is an essential process to confine fibrinolysis to a local level.
D. Fibrin structural modification via thrombin-activatable fibrinolysis inhibitor (TAFI) is a potent attenuator of fibrinolysis. TAFI has specificity for carboxy-terminal arginine and lysine residues that are the binding sites for plasminogen and tPA [11, 22]. Enhanced thrombin generation has been shown to be correlated to increase risk of VTE in trauma patients [23] and may be secondary to the activation of TAFI.
E. A number of proteins with lysine in their surface, including TXA, can bind to plasminogen kringle domains to prevent binding to fibrin.
F. Plasminogen can be converted to angiostatin, an inactive form, by human neutrophil elastase [24].

The exact mechanism of low fibrinolytic activity following trauma is not clear; it is likely multifactorial involving the pathways described above. More recent data suggests that shutdown fibrinolysis patients may be deficient in a pro-fibrinolytic

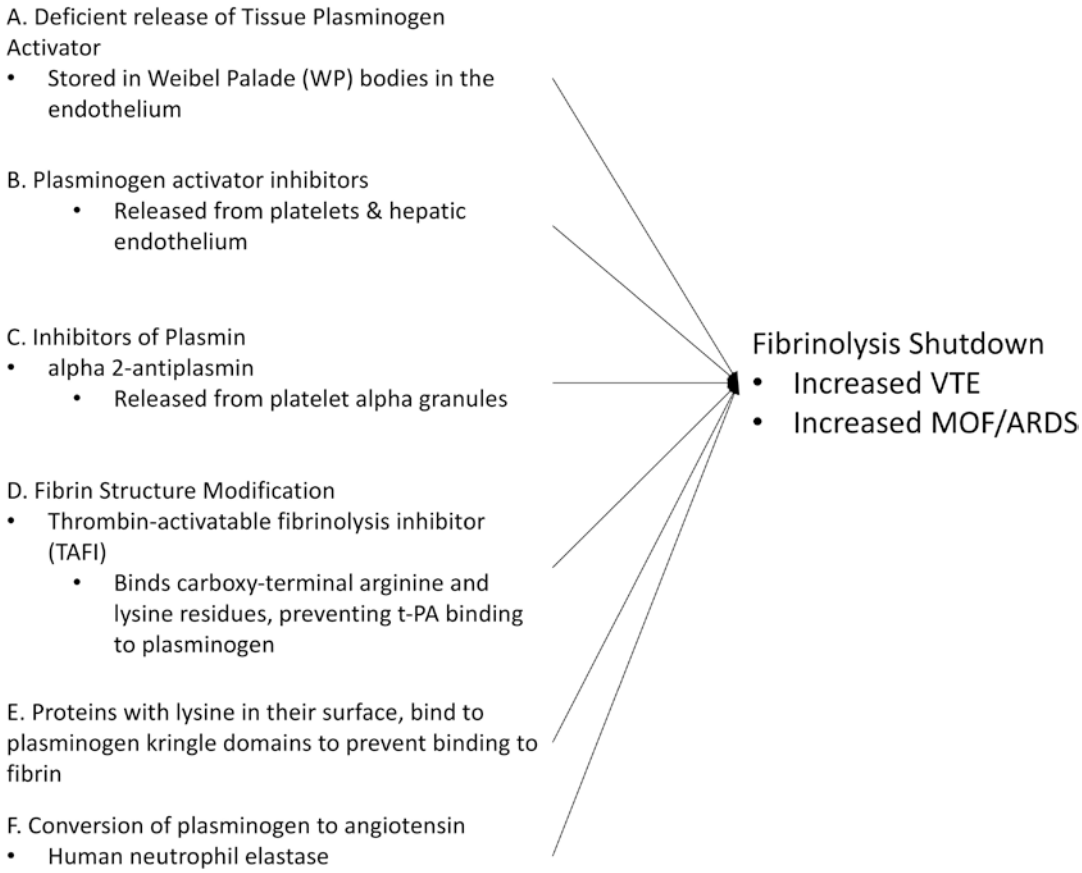


Fig. 31.1 Five ways of decreasing fibrinolytic activity can all contribute to the development of the fibrinolysis shutdown phenotype

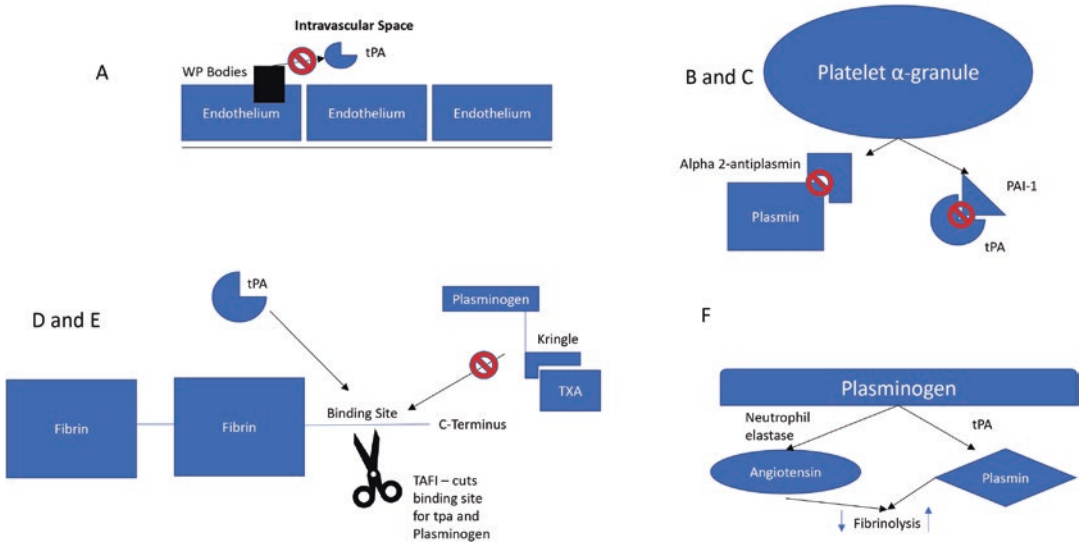


Fig. 31.2 Five ways of decreasing fibrinolytic activity can all contribute to the development of the fibrinolysis shutdown phenotype that is associated with macro- and microvascular complications such as venous thromboembolism and multiorgan failure

mechanism that promotes a physiologic state of fibrinolysis that extends beyond tPA. This is exemplified by the recent report that identified tPA hypersensitive and non-hypersensitive variants of hyper, physiologic, and shutdown and described proteomic differences that may contribute to a fibrinolytic shutdown phenotype as well as effects on overall mortality [25].

Macrovascular Thrombosis, Fibrinolysis Shutdown, and the Effect of Tranexamic Acid

Macrovascular thrombosis can be defined as the presence of venous thromboemboli (DVT and PE), embolic stroke, or myocardial infarction. While early fibrinolysis shutdown is associated with mortality [1, 2], Meizoso et al. also identified persistent fibrinolysis shutdown (persisting longer than 1 week) as an independent predictor of mortality in the severely injured trauma patient [26]. The "Military Application of Tranexamic Acid in Trauma Emergency Resuscitation" (MATTERs) study evaluating the military application of tranexamic acid for the use of injured patients observed that, overall, those who received TXA had a statistically significant increase in rate of DVT [27]. There was also a trend toward increase DVT and PE rate in patients who required a massive transfusion and TXA [27]. In the most recent military evaluation of TXA, it was concluded that there was no overall survival benefit, and its use was associated with a significant increase in thrombotic complications [28]. Obesity has also been found to be a factor associated with increased rates of fibrinolysis shutdown in the trauma population [29]. In this study, a BMI >30 was protective against diminished clot strength and hyperfibrinolysis, and obesity was associated with an increased risk of fibrinolytic shutdown in severely injured patients [29]. It was postulated that this could be a contributing factor to the increased rates of VTE seen in obese patients.

In a pediatric population, the presence of fibrinolysis shutdown at the time of arrival to the emergency department (ED) was associated with a significant increase in the incidence of development of deep vein thrombosis [30]. Furthermore, the group of patients who presented in fibrinolysis shutdown was more likely to be discharged with functional disability. Similarly, another study evaluating the fibrinolytic phenotype in pediatric trauma patients also identified fibrinolysis shutdown as a common postinjury phenotype in this patient population and a high rate of DVT (9.8%) [31]. Furthermore, this group identified that a severe traumatic brain injury (TBI) is associated with sustained impaired clot breakdown [31].

As previously discussed, excess PAI-1 can precipitate a state of fibrinolysis shutdown. For example, several studies have investigated the potential role of alcohol consumption and thromboembolic risk. Spoerke et al. [32] showed that both males and females had increased PAI-1, after consumption of alcohol. In the male population, this was also associated with a decrease in LY30 (clot breakdown). In a study evaluating the effects of alcohol consumption on cardiac risk, Djousse et al. [33] found that individuals who consumed larger amounts of alcohol, more than 14.9 g of alcohol per day (the equivalent of one drink), had increased circulating PAI-1, which they postulated increased the risk of thrombotic complications. Evidence suggests that in acute alcohol intoxication, there is an increase in both tPA and PAI-1 [34, 35]. However, the ratio of PAI-1/tPA is shifted to favor PAI-1 with consumption of an increasing amount of alcohol [34]. Furthermore elevated triglyceride levels resulting from heavy alcohol consumption may further stimulate PAI-1 gene expression, especially in people with a genetic makeup sensitive to PAI-1 [36]. This increased PAI-1 gene expression could result in the inhibition of fibrinolysis and thus increase the risk for acute cardiac events [36]. As alcohol intoxication is present in a large portion of trauma patient, its role in driving fibrinolysis inhibition may have a contributory effect in the development of post-traumatic thrombotic complications. In spinal cord injured patients, alcohol intoxication has been associated with DVT and PE [37]. However, the presence of alcohol in trauma patients has also been found to have the

opposite effect, i.e., an association with lower rates of DVTs [38]. Future study is required, as impairment of fibrinolysis from alcohol may require a secondary hit such as prolonged immobilization to promote VTEs.

Recent studies from the transplant literature implicate fibrinolysis shutdown as a risk factor for VTE. In a study of 49 adult patients undergoing visceral transplantation, fibrinolytic shutdown was the predominant fibrinolytic phenotype. Fibrinolytic shutdown was associated with an increase in both thrombosis and hemorrhage with 18% of patients having an intraoperative thrombosis. A clinically meaningful reduction in incidence of intraoperative thrombosis was noted in visceral transplant recipients who received heparin thromboprophylaxis [39].

While the "Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage" (CRASH-2) trial did not show an increased rate of VTE, there was an association of mortality with late administration of TXA (>3 hours) [40, 41]. These late TXA patients had a paradoxical increase rate of mortality attributable to bleeding. It is important to remember that patients can bleed in low fibrinolytic states. One of the few human clinical studies documenting intravascular clots in organs following severe injury also reported that these patients were clinically coagulopathic [42]. These authors reported no benefit of antifibrinolytics and attributed death due to organ failure, despite the patient continuing to bleed. Other authors have speculated that the post-3-hour cause of bleeding from TXA is due to increased plasmin generation from urokinase [43]. Regardless of the effects, there is an adverse effect of TXA given after 3 hours from injury. This is likely due to a post resuscitation impairment of fibrinolysis as more than 80% of severely injured hypotensive trauma patients have dramatic increases in PAI-1 activity with associated tPA resistance [44]. In this patient population, low fibrinolytic activity between 4 and 12 hours following injury was not associated with increased morbidity or mortality, but those patients that remained in shutdown at 12 and 24 hours had worse outcomes. This reperfusion fibrinolysis shutdown does not appear to be pathologic but rather a physiologic response to severe injury, which has been proposed to be part of the acute-phase response [45]. One argument for empiric TXA use is that the majority of patients will manifest a low fibrinolytic state several hours after injury regardless of their resuscitation strategy and thus, TXA has the potential to attenuate hyperfibrinolysis with minimal effects. However, this concept neglects to take into consideration the effects of TXA on long-term fibrinolytic activity in trauma patients that have been resuscitated.

While the circulating half-life of TXA is several hours, the antifibrinolytic effects can last beyond 24 hours [46]. It has been speculated that this effect is due to TXA binding to plasminogen and remaining in circulation. Recovery from this acquired fibrinolysis shutdown occurs in roughly half of these patients [44]. Those that remain in shutdown are classified as persistent fibrinolysis shutdown, which was previously discussed, in which TXA has been implicated in promoting this scenario [47]. It has been argued that there should be a selective use of TXA in the injured patient as administration to those with a shutdown phenotype may be critical in the pathogenesis of postinjury organ failure and thrombotic complications [48]. Furthermore, patients who present to the hospital with physiologic levels of fibrinolysis, who received TXA, have increased mortality [49]. Finally, a recent study from Myers et al. identified the use of TXA as an independent risk factor for the development of VTE following injury [50]. Recent studies from two separate military groups identified the use of TXA as a risk factor for VTE [28, 51]. One study showed that the use of TXA increased the rate of VTE from 6.8% to 34.5%, while another study revealed that the risk of PE and DVT increased to 182% and 100%, respectively, following the administration of TXA [28, 51]. A 2019 study from Bourdreau et al. evaluating the prehospital use of TXA during aeromedical transport following injury showed that those who received prehospital TXA more commonly presented in fibrinolysis shutdown [52]. Further, whether the patient received prehospital or in-hospital TXA, there

was an increase VTE rate compared to the general trauma population [52].

While the retrospective data suggest a risk of VTE with TXA use, this has not been validated in large randomized trials. It has been appreciated that TXA does not appear to cause thrombotic complications, but rather it increases the clot burden if fibrin is already present [46]. This is likely the more clinically relevant question for concerns with TXA use. The patient population of highest concern would be those patients with blunt carotid vertebral artery injury, which is found in roughly 3% of severely injured trauma patients [53]. Embolic stroke events occur early after injury (<72 hours), and the standard of care is heparin or aspirin therapy [53]. These patients would raise concerns for empiric TXA use.

Overall, the timing of TXA and impact on fibrinolytic shutdown are an important area of future research. It is important to not confuse the three unique states of low fibrinolytic activity following severe injury: (1) acute fibrinolysis shutdown, (2) acquired fibrinolysis shutdown, and (3) persistent fibrinolysis shutdown [10]. Acute fibrinolysis shutdown is a risk factor for persistent shutdown, both of which are associated with increased mortality that occurs days after injury. Acquired fibrinolysis shutdown is a physiologic event 2–12 hours after injury, and recovery from acquired shutdown is associated with favorable outcomes. Appreciation of these different stages will enable a better understanding of postinjury fibrinolysis activity (Fig. 31.3). A knowledge gap to address is if TXA creates a persistent fibrinolytic shutdown state, should these patients be targeted for more intensive VTE screening, and if they require an adjunct to standard chemical and mechanical DVT prophylaxis.

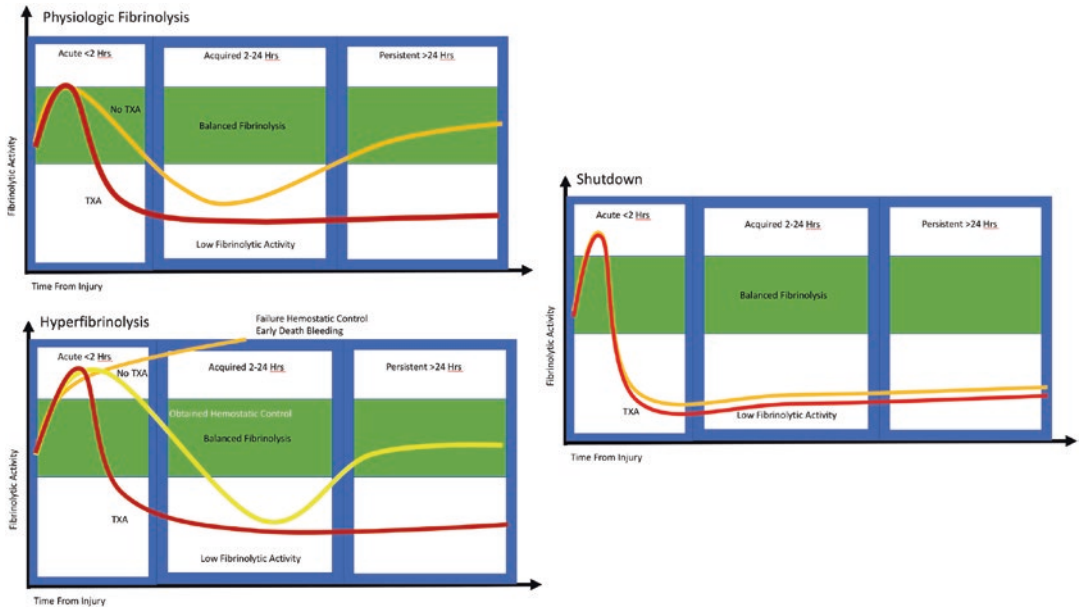


Fig. 31.3 Figure schematically illustrates, in theory, the fibrinolytic activity in different conditions in patients with physiologic fibrinolysis, hyperfibrinolysis, and shutdown fibrinolysis. In patients with physiologic fibrinolysis, the administration of TXA tends to lead to early shutdown fibrinolysis that is persistent. In those that do not receive TXA, fibrinolytic activity is initially balanced, then can decrease to a state of fibrinolysis shutdown, and ultimately recovers to balanced fibrinolysis. In patients with hyperfibrinolysis, there is a similar trend in the presence or absence of TXA. However, there are also patients who do not obtain hemostatic control and die early form bleeding. In the patient cohort with shutdown, both the administration and lack of administration may lead to acute and persistent fibrinolysis shutdown and low fibrinolytic activity

Treatment of Fibrinolysis Shutdown to Prevent Macrovascular Thrombosis

Currently, there are no pharmacologic interventions in use to treat fibrinolysis shutdown (Table 31.1) [54–59]. Furthermore, current interventions have not been uniformly successful in reducing thrombotic complications such as VTE in trauma and surgical patients [60, 61].

However, some studies have illustrated the utility of aspirin and statins as medications as effective at reducing the incidence of VTE [62]. Current guidelines following cardiac surgery recommend the use of aspirin and statins as safe and effective to reduce thrombotic complications [63]. Mechanistically, statins have been shown to reduce the levels of PAI-1, the primary driver of fibrinolysis shutdown, while also increasing plasma fibrin clot permeability and lysability [62, 64, 65]. To investigate this, our group is performing the STAT (Statins and Aspirin in Trauma) trial to evaluate the efficacy of statins and aspirin in reducing the risk of VTE in critically injured patients in the intensive care unit (trials.gov.com #NCT02901067). Enrollment is currently underway and preliminary results are positive in reducing postinjury VTE.

Fibrinolysis Shutdown and Microvascular Thrombosis

Microvascular thrombosis is much more difficult to quantify as it often requires histological evaluation to confirm. However, a surrogate for microvascular thrombosis has been clinical evidence of multiple organ failure. The majority of severely injured trauma patients have impairment of fibrinolysis in the first 12 hours following injury which is associated with increased risk of death from multiple organ failure [1, 2]. This inhibition of fibrinolysis results in microvascular thrombosis and has previously been implicated in the pathogenesis of organ failure [66]. In the post-cardiac arrest syndrome, fibrinolysis shutdown is associated with the development of multiple organ failure. This was suggested to be secondary to higher levels of soluble fibrin as well as increase tPA/PAI-1 complex [67, 68]. In post-cardiac arrest patients, there is an increase in the levels of both neutrophil elastase- and neutrophil-derived fibrin products. Although increased plasmin levels (plasmin-antiplasmin complex) were observed, the results may indicate that t-PA-mediated and neutrophil-mediated fibrinolysis could not overcome PAI-1-induced fibrinolytic shutdown for several days after return of spon-

Table 31.1 Treatment of fibrinolysis

Author	*n*	Population	Treatment	Thrombotic outcome	Intervention vs. control	Fibrinolytic quantification
Knight et al.	128	General surgery	Arm SCD	DVT	32% vs. 14%[a]	ELT[a] POD3
Blamey et al.	60	General surgery	Stanozolol IM 50 mg preoperative	DVT	41% vs. 35%	Fibirn plate lysis[a] POD1
Cuscheieri et al.	50	General surgery	Stanozolol IM 50 mg preoperative	Pulmonary complication	67% vs. 65 %	Fibirn plate lysis[a] POD1
Berridge et al.	27	Vascular surgery	Stanozolol IM 50 mg preoperative and 5 mg PO 6 week	Early graft thrombosis	8% vs. 14%	ELT POD7
Cahan et al.	48	General surgery	Leg SCD and heparin	DVT	0% vs. 0%	PAI-1 activity
Killewich et al.	44	General surgery	Leg SCD and heparin	DVT	0% vs. 0%	PAI-1 activity

Adapted with permission from Moore et al. [10]
Abbreviations: *DVT* deep vein thrombosis, *ELT* euglobulin lysis time, *IM* intramuscular, *PAI-1* plasminogen activator inhibitor 1, *PO* per os, *POD* postoperative day, *SCD* sequential compression device
[a]Statistical difference

taneous circulation leading to the elevation of SOFA scores, which suggests the development of organ dysfunction [68]. Furthermore, fibrinolysis shutdown has been found to be associated with in-hospital death and increased Sequential Organ Failure Assessment (SOFA) scores (suggesting organ dysfunction) in patients with out-of-hospital cardiac arrest [69]. While microvascular thrombosis is difficult to quantify with current technology, ongoing studies are evaluating microfluidics of fibrinolysis to elucidate how the degree of fibrinolysis affects the microvascularity and fluid dynamics in small vessels that may contribute to the microthrombotic complications [70].

Treatment of Fibrinolysis Shutdown to Prevent Microvascular Thrombosis

There are no pharmacologic interventions currently in use to specifically target fibrinolysis shutdown. However, experimental work has evaluated pharmacologic interventions that reduce microvascular thrombosis seen in the acute respiratory distress syndrome (ARDS) [71–75], one of the complications of impaired clot formation and the MOF syndrome [1, 2]. In animal models, Vasquez et al. reported uPA improved blood flow leading to enhanced cardiovascular and pulmonary function compared to controls in a model of septic shock [75]. Furthermore, in swine, Hardaway et al. showed that the administration of t-PA and uPA improved pulmonary function and prevented histologic development of ARDS in both traumatic and septic models of ARDS [73, 74]. Finally, two studies of ARDS secondary to trauma or sepsis illustrated the potential of plasminogen activators at improving pulmonary function [71, 72]. The hypothesis is that disseminated intravascular coagulation (DIC) initiates ARDS by occluding the pulmonary microcirculation with micro clots. The use of plasminogen activators leads to significant improvement in partial pressure of oxygen in arterial blood with no bleeding or aberrations in clotting parameters [71, 72]. These studies suggest that the use of plasminogen activators in selective patients may be of benefit and improve morbidity and mortality associated with microvascular thrombosis associated with states of impaired clot breakdown. Further investigation is clearly needed to identify safe interventions that can reduce or reverse microvascular clot formation associated with fibrinolysis shutdown.

Conclusion

Within the last decade, identifying fibrinolysis phenotypes has become a critical measurement of TEG. Hypofibrinolysis or shutdown fibrinolysis has been shown in some clinical scenarios to increase the risk of macrothrombotic complications such as VTE as well as microthrombotic complications such as multiple organ failure. With the morbidity and mortality of thrombotic sequelae for patients, and the financial burden of the diagnosis and treatment of VTE and multiple organ failure, the identification and appropriate treatment of patients with impaired clot breakdown are critical. We now have potential therapeutic strategies to mitigate shutdown. Finally, antifibrinolytic agents should be used selectively and optimally based on the fibrinolytic status of the patient.

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Congenital and Acquired Hypercoagulable States

32

Joseph Emmerich

Introduction

Venous thrombosis, which includes deep-vein thrombosis (DVT) and pulmonary embolism (PE), affects approximately 1 in 1000 individuals in industrialized countries [1]. The terms "hypercoagulability" and "thrombophilia" are often used to define this condition. Thrombophilia can be congenital (constitutional, genetic, or hereditary) or acquired. Congenital thrombophilia is mainly associated with the occurrence of venous thrombosis, while acquired thrombophilia can be associated with either venous or arterial thrombosis.

In 1856, Virchow described that thrombosis occurs due to stasis of the blood, endothelial injury, and "changes in the composition of blood" (i.e., hypercoagulability). It was only in 1965 that Egeberg published the first case of inherited antithrombin deficiency, which led to an increased interest in thrombophilia, with the description of protein C (PC) and protein S (PS) deficiencies 15 years later [2]. Thrombophilia was initially considered a rare monogenic disorder, but this view was challenged in the mid-1980s with the evidence of more frequent genetic risk factors of thrombophilia, demonstrating that venous thrombosis is a multicausal disease [3]. At this same time, acquired risk factors such as the antiphospholipid syndrome (APS) were described with the occurrence of venous and arterial thrombosis.

We describe here the main congenital and acquired hypercoagulable states associated with thrombosis and their implication in clinical practice according to current evidence and guidelines.

The main risk factors of venous thrombosis are summarized in Table 32.1. Acquired causes of thrombosis, such as trauma or surgery, can trigger the occurrence of venous thrombosis alone or when the basal risk of thrombosis is increased by congenital or hypercoagulable states. Thrombosis must be considered as a multifactorial disease. For further description of hypercoagulability and venous thromboembolism after trauma, please refer to Chap. 31.

Description of Congenital Thrombophilia

Antithrombin Deficiency

Antithrombin (AT) is a 58 kDa plasma protein that regulates coagulation by inhibiting procoagulant serine proteases such as thrombin, activated (a) factor X, and factor IXa. The reaction between AT and thrombin involves the reactive center

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Table 32.1 Main risk factors for venous thrombosis

Acquired risk factors	Genetic risk factors	Other risk factors[a]
Age	Antithrombin deficiency	Hyperhomocysteinemia
History of venous thrombosis	Protein C deficiency	High levels of factor VIII
Surgery	Protein S deficiency	High levels of factor XI
Cancer	Factor V_{Leiden}	High levels of factor IX
Hormonal treatment	Factor II G20210A	High levels of factor VII
Antiphospholipid syndrome	Dysfibrinogenemia	High levels of TAFI
Myeloproliferative disorders	Fibrinogen γ 10034T	Low levels of TFPI
Trauma	Non-O blood group	APCR in the absence of FV_{Leiden}
Plaster cast		
Obesity		

[a]Possible genetic regulation
TAFI thrombin-activatable fibrinolysis inhibitor, *TFPI* tissue factor pathway inhibitor

loop and the protease active site serine and is increased approximately 1000-fold by heparin and other glycosaminoglycans (e.g., heparan sulfate) that are present on the endothelial surface [4]. AT is synthesized by the liver and circulates at a concentration of approximately 2.5 μM; levels are decreased by estrogen and heparin therapy. For further description of AT and its role during physiologic hemostasis as well as during trauma-induced coagulopathy, please refer to Chap. 2.

AT deficiency is transmitted as an autosomal dominant disease (0.02% prevalence in the general population). Among congenital thrombophilias, AT deficiency is undoubtedly the one bearing the highest risk of VTE (approximately 1% per year) [5–7]. There are two types of hereditary AT deficiency. Type I or quantitative deficiency is the most frequent and is characterized by decreased activity and a decreased protein concentration. Type II or qualitative deficiencies are caused by functional defects, protein concentrations being normal or near normal in immunoassays. The dysfunction may affect the reactive site (type II_{RS}) or the heparin-binding site (type II_{HBS}) or both (type II_{PE}—pleiotropic effect) [8]. Homozygosity is mostly present in patients with type II_{HBS}, with the exception of one patient with type II_{PE} deficiency. Venous and arterial thrombosis may occur during infancy in such patients [9].

The ability of AT-deficient plasma to inhibit bovine thrombin and human FXa in the presence of heparin can be assessed by chromogenic heparin cofactor assays. Concentrations of less than 80% (without heparin or estrogen treatment) call for further investigation, although only patients with severe deficiencies (<60%) are at high risk of thrombosis. Chromogenic assays distinguish type II deficiencies, in that type II_{HBS} has normal activity whereas type II_{RS} has low activity; the risk of VTE is very low in type II_{HBS} deficiency [8, 10].

Protein C and Protein S Deficiencies

PC is a vitamin K-dependent protein that is activated at the endothelial surface when thrombin binds to thrombomodulin. This reaction transforms thrombin from a procoagulant enzyme into an inhibitor, by activating PC to activated protein C (APC). In the presence of its cofactor PS, APC degrades activated FV (FVa) and FVIIIa, thereby impeding further thrombin generation [11]. FV inactivation occurs after a rapid cleavage at Arg506, followed by slower cleavage at Arg306 (see below factor V Leiden). PS markedly stimulates the second phase of the inactivation process, by a 20-fold enhancement of Arg306 hydrolysis [12]. The mechanism of FVIIIa inhibition by APC is also biphasic, with cleavage at Arg562 and then at Arg336. FVIIIa inactivation by APC is increased by PS and FV, which act synergistically as cofactors for the reaction.

PC is synthesized by hepatocytes and circulates at a concentration of approximately 70 nM,

with a half-life of approximately 8 h [1]. APC forms inactive complexes with serine protease inhibitors, mainly protein C inhibitor (PCI), but also protease nexin 3, α_1 antitrypsin, and α_2 macroglobulin. Although PS is mainly produced by hepatocytes, it is also detected in endothelial cells and platelets. In the circulation, PS forms inactive complexes with C4b-binding protein (C4b-BP) [13]. Free PS represents approximately 40% of the total circulating level, and only this fraction has APC cofactor activity. PS plasma levels are lower in women younger than 45 years and in those who are pregnant or are using oral contraceptives [14]. For further description of PC and its role during physiologic hemostasis as well as during trauma-induced coagulopathy, please refer to Chap. 6.

PC and PS deficiencies are transmitted, like AT, as autosomal dominant traits. Hereditary PC deficiency was first identified in subjects with a family history of thrombosis. In prospectively studied asymptomatic members of thrombophilic families, the incidence of VTE was approximately 0.5% per patient-year in patients with PC deficiency and between 0.5% and 1.65% in patients with PS deficiency [5–7].

The thrombotic risk associated with PC levels less than 67% was confirmed in a case-control study of unselected patients who developed DVT before 70 years of age, with a relative risk (RR) of approximately 3.1 [confidence interval (CI), 1.4–7.0] [15]. Free PS of less than the 90th percentile of control values is also associated with an increased risk of developing VTE [16]. In heterozygous subjects belonging to families with PC or PS deficiencies, the probability of being free of thrombotic events at 45 years of age is approximately 50%; they are also at risk of recurrent thromboembolic disease in adulthood. Homozygous PS deficiency, or homozygous PC deficiency, are rare thrombophilia phenotypes associated with severe thrombosis, including neonatal skin necrosis and purpura fulminans [17–19].

PC activity is measured after PC activation by Protac with synthetic substrates (amidolytic assays) or by measuring the prolongation of the activated partial thromboplastin time (aPTT) (anticoagulant assays). In type I, quantitative deficiency, the plasma concentration and activity are low; this is the case in most PC deficiencies. Type II, qualitative deficiency, is characterized by normal synthesis of a nonfunctional protein that affects concentration and coagulation assays. Patients with PC concentrations less than 70% may have a hereditary deficiency, although values between 55% and 70% must be considered as borderline [17].

The diagnosis of PS deficiency is complicated by the presence of two plasmatic forms: one is free PS and the second is PS complexed with C4b-BP/PS. PS deficiency is characterized by a low free PS concentration measured by a monoclonal antibody-based immunoenzymatic assay. APC cofactor activity can be evaluated in an aPTT assay after adding diluted plasma to PS-depleted plasma in the presence of purified APC and purified FVa. According to the International Society on Thrombosis and Hemostasis (ISTH) standardization subcommittee, three types of PS deficiency have been defined on the basis of total PS levels, free PS levels, and APC cofactor activity. Type I deficiency is characterized by low total PS and free PS antigen levels, type II deficiency by normal free PS and low APC cofactor activity, and type III PS deficiency by low free PS levels and normal or near-normal total PS levels. Type I and type III deficiencies in fact appear to be two phenotypic expressions of the same genetic disease. The lower normal limit of total and free PS levels is 65% of the level observed in a pool of normal plasmas. However, the reference range in women younger than 45 years is approximately 55% under the same conditions. Therefore, it is recommended to use both the clotting assay and the monoclonal-based immunoassay specific for free PS to screen patients for PS deficiency.

Factor V_{Leiden}

In 1993, Dahlback described three families in whom APC did not yield the expected prolongation of the clotting time in an aPTT assay that defined a new phenotype, called APCR (activated

PC resistance) [20]. APCR was found in more than 15% of patients with DVT and in 2–10% of control subjects [21, 22]. It was then demonstrated that APCR cosegregated with the *FV* gene and with a single base mutation and guanine to adenine at position 1691 of the *FV* gene, which is responsible for the Arg506 Gln mutation known as FV_{Leiden}, affecting one of the APC cleavage sites [23].

FV is a 330-kDa multidomain single-chain glycoprotein, with a plasma concentration of 20 nmol/L (0.007 g/L) [24]. Thrombin and FXa activate FV by a cleavage at peptide bonds at positions 709, 1018, and 1545, thereby releasing the B-domain, which connects the heavy chain (domains A1–A2) to the light chain (domains A3-C1-C2). Upon activation, FVa is formed by the heavy and light chains that are noncovalently associated by a Ca^{2+} ion. FVa is an essential FXa cofactor; its presence in the prothrombinase complex enhances the rate of prothrombin activation into thrombin by 10^3- to 10^5-fold. Downregulation of the procoagulant activity of FVa is accomplished through its inactivation by APC at positions Arg306, Arg506, and Arg679. Cleavage at Arg506 is essential for optimal exposure of cleavage sites Arg306 and Arg679 but results in partial inactivation of FVa (approximately 40% of procoagulant activity remains). Therefore, any defect on one or more of these three cleavage sites (i.e., Arg506, Arg306, and Arg679) may potentially affect inactivation by APC. FVa inactivation is enhanced by protein S.

FV_{Leiden} results in a substantially reduced anticoagulant response to APC, because FV_{Leiden} is inactivated about ten times slower than normal FV. This impairment of FVa inactivation increases thrombin generation and explains more than 90% of clinical APCR phenotypes. The fact that FV_{Leiden} is a much less active cofactor of APC than wild-type FV for FVIIIa inactivation contributes also to the APCR phenotype in subjects with FV_{Leiden}. Clinical states with low APC sensitivity that are not caused by FV_{Leiden} may also be acquired, as is the case during pregnancy, with the use of oral contraceptives, or in patients with lupus anticoagulant or high levels of FVIII.

Most cases of APCR are caused by FV_{Leiden}, but two other FV mutations affect the Arg306 cleavage site. Arg306 replaced is by Gly in $FV_{Hong\ Kong}$, and Arg306 is replaced by Thr in $FV_{Cambridge}$. $FV_{Hong\ Kong}$ is prevalent (approximately 5%) among the Chinese in Hong Kong, but neither of these mutations are associated with an increased risk of developing venous thrombosis [25]. FV Ile359Thr (FVLiverpool) and FV Glu666Asp are two rare mutations also associated with thrombosis and APCR [26].

The prevalence of FV_{Leiden} is high in populations of white descent, but low in native populations of Asia, Africa, and Australia [27]. All FV_{Leiden} alleles are carried by the same haplotype, leading to the inference that the mutation occurred only once and spread by a founder effect. The estimated time of the mutation is approximately 30,000 years, implying that it took place after the out-of-Africa divergence that occurred approximately 100,000 years ago [28]. Its spread among Whites and its high prevalence suggest that FV_{Leiden} is associated with a survival advantage, such as a decrease in severe bleeding after delivery.

The frequency of FV_{Leiden} in white populations is approximately 5% for Caucasians, being present in 15–25% of Caucasian patients with DVT, and the risk of DVT in heterozygous carriers is approximately fivefold higher than in a control population [29]. The association with pulmonary embolism is much weaker than with deep vein thrombosis [30, 31]. Familial and case-control studies show that FV_{Leiden} is a milder thrombophilic state than heterozygous AT, PC, or PS deficiency [5–7]. The absolute risks of VTE events in subjects with AT, PC, and PS deficiency are between 0.5% and 1.5% per year, compared to only 0.1–0.3% per year in heterozygous carriers of FV_{Leiden}.

The prevalence of FV_{Leiden} homozygosity in the general population is approximately 1 in 2500. The thrombotic complications are far less severe than in homozygous PC and PS deficiencies [32]. FV_{Leiden} homozygosity was found in 4.1% of 1200 consecutive patients with juvenile VTE [33]. In a pooled analysis of eight case-control studies, we found FV_{Leiden} homozygosity in 1.3% of 2310 cases and 0.13% of 3204 controls [28]. In homo-

zygotes, the risk of venous thrombosis is increased 30- to 140-fold, yet an additional environmental or genetic risk factor is often present at the first thrombotic event [32, 33].

Two types of tests can be used for diagnosing APCR: the functional APCR assay and the genetic FV_{Leiden} test. The functional assay is based on aPTT prolongation after the addition of purified APC and is expressed as an APC sensitivity ratio: APTT(+APC)/APTT(−APC). A low APC sensitivity ratio defines APCR. Second-generation APCR tests, which use dilution of test plasma into FV-deficient plasma, have very good specificity and can even be used to test patients taking warfarin [34]. It is mandatory to confirm a positive APCR test by direct detection of FV_{Leiden}. The advantage of genetic testing is that it avoids ambiguous results and determines heterozygous/homozygous status in patients with a low APC sensitivity ratio.

The FII (Prothrombin) G20210A Mutation

Prothrombin has procoagulant, anticoagulant, and antifibrinolytic activities after its activation into thrombin by the prothrombinase complex [1]. Thrombin acts by activating factors XIII, XI, and VIII, V, PC, and the thrombin-activatable fibrinolysis inhibitor (TAFI) and by cleaving fibrinogen to fibrin. Prothrombin is a 72-kDa multidomain single-chain vitamin K-dependent glycoprotein [35]. Prothrombin activation is mediated by FXa, which cleaves prothrombin at Arg271–Thr272 and Arg320–Ile321 to release the catalytic domain from the carboxy-terminal domain. Prothrombin activation is accelerated approximately 300,000-fold in the presence of FVa, phospholipids, and Ca^{2+}. The prothrombin gene is 21 kb in length and is encoded by chromosome 11 (position 11p11-q12); it comprises 14 exons separated by 13 introns, with the 5′ and 3′ untranslated regions that may play regulatory roles in gene expression.

By extensively screening the prothrombin genes of 28 families with unexplained venous thrombosis, Poort et al. found one heterozygous nucleotide transition (G to A) at position 20210 in the 3′ untranslated region in 5 probands (18%) [36]. This mutation is associated with significantly higher prothrombin levels in heterozygotes than in noncarriers and is an independent risk factor for thrombosis, leading to increased thrombin generation [37].

The prevalence of the FII mutation is high in populations of white descent but low or nil in Asians, American Indians, and African Americans [38]. Similarly to FV_{Leiden}, a founder effect explains the high prevalence of the factor II G20210A mutation in Caucasians [39].

The estimated frequency of FII G20210A in white populations is around 2–3% [29]. This mutation is more common in Southern than in Northern Europe, a gradient opposite to that of FV_{Leiden} [36]. The risk of DVT is increased by threefold to fourfold in heterozygous carriers of FII G20210A compared to noncarriers. In the rare FII G20210A homozygotes, the risk of thrombosis is only moderately increased, often associated with other genetic or acquired risk factors [40].

Although FII G20210A heterozygotes have 30% higher prothrombin levels than noncarriers on average, this phenotype cannot be used to identify carriers of the mutation because of a large overlap of prothrombin levels. To check for the mutation, genetic screening after PCR amplification is the only reliable method.

Other Genetic Risk Factors

Some more rare genetic risk factors of thrombosis have been described. They have drawn much less interest in clinical practice, and they are not part of routine thrombophilia testing.

Blood Group Non-O

It was known since 1969 that non-O blood groups are associated with an increased risk of venous thrombosis [41]. O blood group is associated with decreased levels of factor VIII and vWF, due to increased clearance of vWF [41]. A twofold increased risk for all non-OO genotypes, with a synergistic interaction with FV_{Leiden}, was con-

Table 32.2 Main features of hereditary thrombophilia

Protein affected	Antithrombin	Protein C	Protein S	Factor V	Factor II
Gene location	1q23-25	2q13-14	3p11	1q21-22	11p11-q12
Type of mutation(s)	Loss-of-function			Gain-of-function	
	Private mutations			Arg506Gln	G20210A
Frequency in the general population %	0.02	0.2–0.4	0.7–2.3	2–10	2–4
Type of assay	Heparin cofactor activity against FXa	Clotting assay or amidolytic assay	Clotting assay or immunoassay for free PS	APCR (second-generation aPTT-based assay) or FV genotyping	FII genotyping
Functional effect	Thrombin and FXa inhibitor	Reduce thrombin generation by inactivating FVa and FVIIIa		FV variant resistant to APC inactivation	Increase the circulating FII concentration
Risk of VTE	×10	×4–5	×4–5	×4–5	×3–4

PS protein S, *APCR* activated protein C resistance, *aPTT* activated partial thromboplastin time, *APC* activated protein C, *VTE* venous thromboembolism

firmed in the Leiden Thrombophilia Study [42]. The attributable risk of venous thrombosis to the non-O blood group is high due to the magnitude of the frequency.

X-Linked Thrombophilia (Factor IX Padua)

If all genetic causes of thrombophilia were reported to date to be autosomal dominant, a recent report described X-linked thrombophilia due to a Leu for Arg mutation in the factor IX gene. This mutation was found as a gain-of-function of factor IX, as the male proband had a ratio of activity to antigen of 8 [43]. This mutation was only found in one family and does not play a role in the epidemiology of the disease. On the other hand, it is an excellent paradigm defining thrombophilia as the counterpart of hemophilia (see section "Introduction"). Furthermore, it demonstrates that rare mutations in candidate genes could also play a role in unexplained familial thrombophilia.

A Prothrombin Mutation Conveying Antithrombin Resistance

Recently, another exceptional prothrombin mutation was reported, a substitution of arginine for leucine at position 596. This prothrombin mutation had moderately lower activity than wild-type thrombin, but mainly its inactivation by antithrombin was substantially impaired. It thus results in an increase in thrombin generation explaining the thrombophilia observed in this family [44].

Table 32.2 summarizes the main genetic hypercoagulable states. Other polymorphisms in candidate genes have been described but are not described here, as their relation with the occurrence of VTE has not been demonstrated.

Clinical Consequences of Congenital Thrombophilia

There is no consensus on the subsets of subjects who qualify for thrombophilia screening, the types of laboratory test to use, or the clinical treatment of patients with thrombophilia. When to screen for thrombophilia depends mainly from the knowledge concerning the associations between thrombophilia and its clinical consequences. Congenital thrombophilia is mainly associated with venous thrombosis. The most salient recommendations for testing are summarized in Table 32.3. Finally, testing for thrombophilia would be useful if we were able to identify patients who are particularly prone to recurrence; however, this is not always the case as the risk of recurrence is not increased for FV_{Leiden} or FII

Table 32.3 Recommendations concerning screening for thrombophilia

1. *Thrombophilia screening is recommended in patients with*:
A history of recurrent VTE
A first VTE at younger than 50 years of age
A first unprovoked VTE at any age
A first VTE at an unusual anatomic site, such as the upper limb, cerebral, mesenteric, portal, or hepatic
A first VTE related to pregnancy, the puerperium, contraceptives, or hormone replacement therapy
Women with two or more unexplained pregnancy losses
2. *Testing for thrombophilia is controversial in*:
Young women smokers (age < 50 years) with a myocardial infarction
Elderly patients (age > 50 years) with a first provoked VTE event in the absence of cancer or an intravascular device
A first VTE related to SERMs or tamoxifen
Selected cases of women with unexplained severe preeclampsia, placental abruption, or intrauterine growth retardation
3. *Testing for thrombophilia may be indicated in*:
Asymptomatic adult family members of probands with known coagulation inhibitor deficiency and maybe in FV_{Leiden} families (especially those with a strong family history of thrombosis at a young age)
Asymptomatic female family members who are pregnant or are considering oral contraceptives or pregnancy
4. *Thrombophilia testing is not recommended*:
As a general population screen
As a routine initial test prior to or during oral contraceptive use, hormone replacement therapy, or SERM therapy
As a prenatal test, newborn initial test, or routine test in asymptomatic prepubescent children
As a routine initial test in patients with arterial thrombotic events; however, testing can be considered in certain unusual situations, such as in patients with unexplained arterial thrombosis without atherosclerosis or in young patients

VTE venous thromboembolism, *SERMs* selective estrogen receptor modulators, FV_{Leiden} factor V_{Leiden}

G20210A, even in homozygous or double heterozygous states [45, 46].

The NICE guidelines, issued in 2012, concerning the management of VTE and the role of thrombophilia testing made the following recommendations concerning thrombophilia screening (www.nice.org.uk/guidance/cg144). These guidelines are for main indications and do not take into consideration detailed situations.

1. Do not offer thrombophilia testing to patients who are continuing anticoagulation treatment.
2. Consider testing for hereditary thrombophilia in patients who have had unprovoked DVT or PE and who have a first-degree relative who has had DVT or PE if it is planned to stop anticoagulation treatment.
3. Do not offer thrombophilia testing to patients who have had provoked DVT or PE.
4. Do not routinely offer thrombophilia testing to first-degree relatives of people with a history of DVT or PE and thrombophilia.

Probably, the most evidence-based and detailed information concerning thrombophilia screening and clinical consequences are those from the British Society of Haematology [47]. A partial summary is reproduced below that are applicable to trauma patients (criteria used to quote levels and grades of evidence are indicated in bracket):

- Initiation and intensity of anticoagulant therapy following a diagnosis of acute venous thrombosis should be the same in patients with and without heritable thrombophilia (1B).
- Indiscriminate testing for heritable thrombophilias in unselected patients presenting with

a first episode of venous thrombosis is not indicated (1B).
- Decisions regarding duration of anticoagulation (lifelong or not) in unselected patients should be made with reference to whether or not a first episode of venous thrombosis was provoked or not, other risk factors, and risk of anticoagulant therapy-related bleeding, regardless of whether a heritable thrombophilia is known (1B).
- Testing for heritable thrombophilias in selected patients, such as those with a strong family history of unprovoked recurrent thrombosis, may influence decisions regarding duration of anticoagulation (C). It is not possible to give a validated recommendation as to how such patients should be selected.
- Testing is not recommended in unselected patients with upper limb venous thrombosis (1B).
- Testing is not recommended in patients with central venous catheter (CVC)-related thrombosis (1C).
- Testing for heritable thrombophilia after a first episode of cerebral vein thrombosis (CVT) has uncertain predictive value for recurrence (C). Decisions regarding duration of anticoagulant therapy in relation to the results of testing are not evidence-based.
- Testing is not indicated in patients with retinal vein occlusion (1B).
- Testing for heritable thrombophilia after a first episode of intra-abdominal vein thrombosis has uncertain predictive value for recurrence (C). Decisions regarding duration of anticoagulant therapy in relation to the results of testing are not evidence-based.
- Neonates and children with purpura fulminans should be tested urgently for protein C and S deficiency (1B).
- A variety of functional methods may be required to identify specific severe type 2 functional defects when levels of protein C or S are not <5% (1B).
- It is suggested that adults who develop skin necrosis in association with oral vitamin K antagonists (VKAs) are tested for protein C and S deficiency after VKA treatment is withdrawn (2B).
- Case finding of asymptomatic relatives with low-risk thrombophilia, such as F5G1691A (FVR506Q, factor V Leiden) or F2G20210A, is not indicated (1B).
- Case finding of asymptomatic relatives with high-risk thrombophilia, such as deficiency of antithrombin , protein C, or protein S, should only be considered in selected thrombosis-prone families (1B). If testing is performed, the risks, benefits, and limitations of testing should be discussed in the context of explained inheritance and disease risk. It is not possible to give a validated recommendation as to how such patients and families should be selected.
- Case finding for very rare homozygosity or compound heterozygous heritable thrombophilia is not indicated as because these defects are so rare, they are not predicted by family history, and the risk of unprovoked thrombosis is low (2C).
- If a first-degree relative with venous thrombosis has not been tested, then suggest that women consider an alternative contraceptive or transdermal hormone replacement therapy (HRT). Testing for heritable thrombophilia will provide an uncertain estimate of risk and is not recommended (1C).
- If a first-degree relative with venous thrombosis has been tested and the result is negative, then suggest that a woman consider an alternative contraceptive or transdermal HRT. Testing for heritable thrombophilia will provide an uncertain estimate of risk and is not recommended (1C).
- If a first-degree relative with venous thrombosis has been tested and the result is positive, then suggest that women consider an alternative contraceptive or transdermal HRT before offering testing as a negative test result does not exclude an increased risk of venous thrombosis. Testing for heritable thrombophilia may assist counseling of selected women particularly if a high risk thrombophilia has been identified in the symptomatic relative (C).
- Women should be assessed for risk of pregnancy-associated venous thrombosis primarily in relation to clinical risk factors (1B).
- Most pregnant women with a previous unprovoked venous thrombosis (1B) or pregnancy

or combined oral contraceptive (COC)-related thrombosis (2C) will qualify for thromboprophylaxis on clinical risk alone, and so testing for heritable thrombophilia is not required.
- Pregnant women with a previous event due to a major provoking factor, e.g., surgery or major trauma, would not usually require prophylaxis or testing (2B).
- Pregnant women with a previous event due to a minor provoking factor, e.g., travel, should be tested and considered for prophylaxis if a thrombophilia is found (2C).
- In the asymptomatic pregnant woman with a family history of venous thrombosis, testing is not required if the clinical risks alone are sufficient to result in thromboprophylaxis (2C).
- It is suggested that asymptomatic pregnant women with a family history of venous thrombosis be tested if an event in a first-degree relative was unprovoked or provoked by pregnancy, COC exposure, or a minor risk factor (2C). The result will be more informative if the first-degree relative has a known thrombophilia.
- Antithrombotic therapy should not be given to pregnant women with a history of pregnancy complications based on testing for heritable thrombophilia. Randomized controlled trials with a no-treatment or placebo arm in women with a history of pregnancy complications are in progress. If these studies indicate a benefit in women with pregnancy complications and heritable thrombophilia, as compared with women without thrombophilia, only then would there be a rational basis for recommending that antithrombotic therapy is given to pregnant women with a history of pregnancy complications based on testing for heritable thrombophilia.
- Testing asymptomatic women before assisted conception and those with ovarian hyperstimulation syndrome is not indicated (1B).
- Thrombophilia screening of hospitalized patients to identify patients at risk of hospital-acquired venous thrombosis is not indicated (1A).
- All hospitalized patients should be assessed for risk of venous thrombosis regardless of heritable thrombophilia based on a clinical risk assessment (1B). The presence of a previously known heritable thrombophilia may influence the assessment of risk.
- Testing for heritable thrombophilia is not indicated in patients with arterial thrombosis (1B).
- It is suggested that testing for heritable thrombophilia is not indicated in children with stroke (2C).

For patients with trauma or undergoing surgery, prophylaxis recommendations are similar for patients with or without thrombophilia. Patients with history of VTE and a thrombophilic defect need short-term thromboprophylaxis to cover periods of increased thrombotic risk, for example, surgery, trauma, plaster casts, or immobilization. Affected asymptomatic relatives of patients with thrombophilia merit consideration for short-term thromboprophylaxis to cover periods of increased thrombotic risk.

Finally, in recent years, it clearly arises that if undoubtedly congenital thrombophilia allows a better understanding of the physiopathology of VTE, the clinical consequences of thrombophilia screening are limited in the majority of cases [48, 49]. For this reason, careful selection of patients eligible for screening is recommended, and expert physicians in the field should analyze the data to provide adapted care in rare cases and to perform genetic screening within families, when requested.

Acquired Thrombophilia

Acquired thrombophilia is mainly represented by two general conditions: the antiphospholipid syndrome and myeloproliferative neoplasms. Cancer is associated with a well-known increased risk of venous thrombosis and of recurrence despite oral anticoagulation, but it is not discussed here.

Antiphospholipid Syndrome

The antiphospholipid syndrome (APS) is an autoimmune systemic thrombophilic disorder characterized by recurrent thrombosis and/or obstetrical morbidity in the presence of at least

Table 32.4 International consensus criteria for the diagnosis of APS (revised classification of 2006)

Clinical criteria
1. *Vascular thrombosis*
One or more clinical episodes of arterial, venous, or small-vessel thrombosis, in any tissue or organ
Thrombosis should be supported by objective validated criteria (unequivocal findings of appropriate imaging studies or histopathology). For histopathological support, thrombosis should be present without substantial evidence of inflammation in the vessel wall
2. *Pregnancy morbidity, defined by one of the following criteria*:
(a) One or more unexplained deaths of a morphologically healthy fetus at or beyond the tenth week of gestation, with healthy fetal morphology documented by ultrasound or direct examination of the fetus
(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of (1) eclampsia or severe preeclampsia defined according to standard definitions or (2) recognized features of placental insufficiency[a]
(c) Three or more unexplained consecutive spontaneous abortions before the tenth week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded
Laboratory criteria
3. *Lupus anticoagulant (LA)* present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies)
4. *Anticardiolipin (aCL)* antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e., >40 GPL or MPL or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA
5. *Anti-β 2 glycoprotein-I antibody* of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met

[a](1) Abnormal or non-reassuring fetal surveillance test(s), e.g., a nonreactive nonstress test, suggestive of fetal hypoxemia; (2) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g., absent end-diastolic flow in the umbilical artery; (3) oligohydramnios, e.g., an amniotic fluid index of 5 cm or less; or (4) a postnatal birth weight less than the tenth percentile for the gestational age

one persistent antiphospholipid antibody [50, 51]. Several other clinical manifestations can be associated with APS. The clinical and biological criteria for the diagnosis of APS were updated in 2006 and are indicated in the Table 32.4 [52]. The diagnosis of APS is considered if at least one of the clinical criteria is associated with at least one of the laboratory criteria.

APS can be primary or secondary to systemic lupus erythematosus (SLE) . In SLE, approximately one-third of patients have antiphospholipid antibodies and approximately half of those (10–15%) will have complications due to APS.

APS is also associated with many other clinical manifestations, also known as "noncriteria clinical manifestations," that are listed in Table 32.5. The most severe but rare manifestation of APS is the "catastrophic antiphospholipid syndrome." The severity of this manifestation is related to multiorgan failure related to widespread small-vessel thrombosis characterized histologically by acute thrombotic microangiopathy. Criteria for the diagnosis of catastrophic APS have been edicted [53]. The definite diagnosis requires all four criteria:

1. Evidence of involvement of three or more organs, systems, and/or tissues
2. Development of manifestations simultaneously or in less than 1 week
3. Confirmation by histopathology of small-vessel disease occlusion in at least one organ or tissue
4. Laboratory confirmation of the presence of antiphospholipid antibodies

A high index of suspicion of this diagnosis of catastrophic APS requires aggressive and immediate treatment in order to reduce the mortality, which is approximately 50%.

Table 32.5 Other clinical manifestations, "noncriteria clinical manifestations," associated with the antiphospholipid syndrome (some of these clinical manifestations can be secondary to unusual arterial occlusions)

1. *Neurologic manifestations*
Migraines, seizures, chorea, Guillain-Barré syndrome (transverse myelitis), transient global amnesia, dementia, peripheral neuropathy, orthostatic hypotension
2. *Cardiovascular manifestations*
Premature atherosclerosis, heart valve disease (valve thickening/vegetations)
3. *Pulmonary manifestations*
Pulmonary arterial hypertension, acute diffuse alveolar damage
4. *Hepatic and gastrointestinal manifestations*
Esophageal necrosis, pancreatitis, colonic ulceration, acalculous acute cholecystitis, mesenteric venoocclusive disease, primary biliary cirrhosis, Budd-Chiari syndrome
5. *Renal manifestations*
Nephropathy, renal artery stenosis, minimal change/focal segmental glomerulonephrosis, membranous nephropathy, mesangial C3 nephropathy, pauci-immune crescentic glomerulonephritis
6. *Skin manifestations*
Leg ulcers, digital gangrene, skin necrosis, livedo reticularis, erythematous macules, purpura ecchymoses, painful nodules, subungual splinter hemorrhages, anetoderma, discoid lupus erythematosus, cutaneous T-cell lymphoma
7. *Ophthalmological manifestations*
Retinal vein or arterial occlusion, amaurosis fugax, optic neuropathy
8. *Hematological manifestations*
Thrombocytopenia, hypoprothrombinemia, acquired APC resistance, protein S deficiency, acquired von Willebrand syndrome, inhibitors to specific coagulation factors, leukopenia, autoimmune hemolytic anemia
9. *Other manifestations*
Acute adrenal insufficiency due to bilateral adrenal hemorrhage, osteonecrosis, and acute sensorineural hearing loss:

The biological diagnosis of APS requires the presence of antiphospholipid antibodies. Both lupus anticoagulant (LA) and anticardiolipin (aCL) antibodies of isotype G (IgG) and isotype M (IgM) were initially part of the diagnosis. In the revised criteria, anti-β2 glycoprotein I (anti-β2GPI) antibody of IgG and IgM isotype were included. The presence of at least one antiphospholipid antibody (with at least one clinical criterion) is necessary for the diagnosis of APS.

Lupus anticoagulants are detected by inhibition of phospholipid-dependent coagulation reaction. They can be suspected by a spontaneous increase of the activated partial thromboplastin time (aPTT), which is not corrected by mixture with normal plasma. More specific tests must be used to screen for the presence of LA: the dilute Russell's viper venom time (dRVVT) is a sensitive test to detect the presence of inhibitor of phospholipid-dependent coagulation, but false positives exist in case of anticoagulation or other anticoagulation abnormalities. Different aPTT tests with LA-sensitive reagent versus LA-insensitive reagent and their correction with hexagonal phase array phospholipids or frozen washed platelets (neutralization procedures) can also be used.

The anticardiolipin immunoassay is expressed in IgG phospholipid units (GPL) or IgM phospholipid units (MPL), with one unit corresponding to the cardiolipin-binding activity of 1 μg/ml of affinity-purified antibodies. This test has a high sensitivity but a poor specificity mainly in asymptomatic patients, as aCL antibodies can also be associated with infectious diseases. For this reason, positive aCL antibodies as well as LA or anti-β2GPI always need to be confirmed 12 weeks after the initial screening.

Triple positivity for LA, aCL, and anti-β2GPI antibodies is strongly associated with the occurrence of the clinical symptoms of APS. Anti-β2GPI is the main target of aPL antibodies and is usually present in association with aCL antibodies, but some patients can have isolated anti-β2GPI antibodies. High levels of antibodies or LA are also more strongly associated with com-

plications. Antiphospholipid antibodies bind to endothelial cells, platelets, and monocytes in a β2GPI-dependent manner. Several pathogenic mechanisms explain the occurrence of thrombosis [50, 54]:

- Inhibition of endogenous anticoagulant and fibrinolytic mechanisms
- Activation of platelets
- Injury and activation of endothelial cells
- Complement activation

Long-term anticoagulation with oral anticoagulants is the cornerstone for treatment of APS once thrombosis has occurred. Intensity of antivitamin K is recommended at the usual doses (INR between 2 and 3), but in some severe cases recurrence can be observed and higher anticoagulation levels are then necessary [54, 55]. The new oral anticoagulants (direct inhibitors of thrombin or factor Xa) are of potential interest for long-term treatment, but a recent randomized study was stopped prematurely, due to a large excess of events in triple positive for LA, aCL, and anti-β2GPI antibodies in patients treated with rivaroxaban compared to antivitamin K [56]. There can be one exception for anticoagulation in thrombosis associated with APS: it is the secondary prevention of stroke in patients without other history of thrombosis. In the APASS study, a randomized trial comparing warfarin (INR between 1.4 and 2.8) to aspirin (325 mg) in patients with APS and ischemic stroke, no differences were observed in the recurrence rates after a follow-up of 2 years [57]. This negative result could be explained by the fact that the majority of patients had low or moderate levels of LA or ACL antibodies and by the low intensity of warfarin treatment and also because APS could not be ascertained in this population as repeat testing was not performed.

Hydroxychloroquine (HCQ) is a synthetic antimalarial drug currently used in SLE. It has anti-inflammatory and immunomodulatory properties without causing immunosuppression. It increases lysosomal pH, interferes with antigen processing, and modulates the immune response mediated by toll-like receptor 9. Furthermore, HCQ has an antithrombotic effect by inhibition of platelet aggregation, by reduction of the attachment of anti-β2GPI complexes to phospholipids and cells, by reversing the binding of antiphospholipid antibodies to syncytiotrophoblasts, and by restoring annexin 5 expression. For these reasons, HCQ is a well-established drug used for treatment of SLE, and it has been demonstrated that in secondary APS, it reduces arterial and venous thrombosis events [55]. A recent prospective non-randomized study compared anticoagulation alone versus HCQ (400 mg daily) plus anticoagulation in primary APS. Six recurrent thrombotic events were observed in the 20 patients treated with anticoagulation alone, compared to none in the HCQ plus anticoagulation group [58]. A major limitation of this study is that only venous thrombosis events were included, and its results do not represent the effects of this treatment modality on arterial thrombotic events.

Statins, beside their hypocholesterolemic and preventive effects on coronary artery disease, also display pleiotropic and anti-inflammatory properties [54, 55]. Since statins have been beneficial in this setting, clinical studies remain necessary to fully establish their role in APS.

For the treatment of the catastrophic APS, a life-threatening disease, aggressive treatment is required, which is a combination of anticoagulation, glucocorticoids, plasma exchange, cyclophosphamide, intravenous immunoglobulins, and antiplatelet agents. New therapeutic approaches have been tested in small series of patients using rituximab (anti-CD20 antibody), eculizumab (inhibitor of complement terminal pathway), and defibrotide.

In pregnant women, the goal of treatment is the prevention of adverse maternal and fetal outcomes such as maternal thrombosis, fetal loss, eclampsia, placental insufficiency, fetal growth restriction, and preterm birth. Subcutaneous low-molecular-weight heparin (LMWH) and low-dose aspirin are the treatment of APS in pregnancy with introduction of LMWH during the first trimester and aspirin prior to conception due to its beneficial effect on early stage of implantation [51]. There is no more room for glucocorticoids or venous immunoglobulins in the prevention of APS-related complications associated with pregnancy.

Myeloproliferative Neoplasms

Myeloproliferative neoplasms (MPN) comprise three classic entities: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), which are clonal expansion of an abnormal stem/progenitor cell (BCR-ABL negative) [59–61]. They are associated with the JAK2 (V617F) mutation in exon 14, which is found in >95% of PV and around 60–70% of ET and PMF patients. In patients with ET or PMF without JAK2 mutations, somatic mutations in the endoplasmic reticulum chaperone CALR (Calreticulin) are also frequent, associated with 15–25% of ET or PMF [62]. Other mutations have also been identified in MPN, like myeloproliferative leukemia virus oncogene (MPLW515L is an acquired mutation that induces constitutive activation of the JAK-STAT pathway), or in exon 12 of JAK2. MPN are rare diseases, as for each of them, their yearly incidence is between 1 and 2/100,000. The usual median age at diagnosis is around 70 years, but around 20% of cases occur in younger individuals. Their natural history is a predisposition to thrombo-hemorrhagic complications (for PV and ET) but also the risk to transform into myelofibrosis and acute leukemia. It is not rare that the diagnosis is made after an arterial or venous thrombotic event, based on anomalies found in the systematic hemogram performed at this occasion or on hemogram performed in asymptomatic patients.

Endogenous in vitro erythroid or megakaryocyte colony formation, in the absence of growth factors, is a characteristic of MPN. Erythropoietin-independent proliferation is observed mainly in PV but also in ET and PMF.

The diagnosis of PV, ET, and PMF is based on the World Health Organization diagnostic criteria from 2008, summarized in Table 32.6.

PV must be suspected in the presence of hemoglobin levels above 18.5 g/dl in men and 16.5 g/dl in women. The second major criterion is the presence of the JAK2 V617F mutation or JAK2 exon 12 mutation. Three minor criteria are bone marrow trilineage proliferation, subnormal levels of erythropoietin (EPO), and endogenous erythroid colony growth. In clinical practice when the diagnosis of PV is suspected, EPO measurement and search for JAK2 V217F must be performed:

- If EPO is subnormal and JAK2 V217F mutation is positive, the diagnosis is done.
- If EPO is normal or elevated and JAK2 V217F mutation is negative, the diagnosis of PV is unprobable.

Table 32.6 Criteria for polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)

	Major criteria	Minor criteria
PV	1. Hb > 18.5 g/dl for men and 16.5 g/dl for women	1. BM trilineage proliferation
	2. Presence of JAK2V617F or JAK2 exon 12 mutation	2. Subnormal EPO levels
		3. Endogenous erythroid colony growth
ET	1. Platelet count > 450 × 10^9/L	
	2. Megakaryocyte proliferation with large and mature morphology	
	3. Not meeting WHO criteria for CML, PV, PMF, myelodysplasia, or other myeloid neoplasm	
	4. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive thrombocytosis	
PMF	1. Megakaryocyte proliferation and atypia accompanied by either reticulin or collagen fibrosis	1. Leukoerythroblastosis
	2. Not meeting WHO criteria for CML, PV, PMF, myelodysplasia, or other myeloid neoplasm	2. Increased serum LDH level
	3. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive marrow fibrosis	3. Anemia
		4. Palpable splenomegaly

BM bone marrow, *Hb* hemoglobin, *EPO* erythropoietin, *CML* chronic myeloid leukemia, *LDH* lactate dehydrogenase

- If EPO is subnormal and JAK2 V217F mutation is negative, screening for JAK2 exon 12 mutation and bone marrow biopsy are required.

The diagnosis of PV must be suspected in the presence of clinical symptoms often insidious and nonspecific such as pruritus increased by water, reddened face, headaches, dizziness, weakness, fatigue, unexplained weight loss, excessive sweating, double or blurred vision, and gouty arthritis. Nowadays, diagnosis is often suspected after reading a hemogram performed for another reason or at the time of a complicated event such as arterial or venous thrombosis or more rarely hemorrhage.

The diagnosis of ET must be suspected by nonspecific symptoms such as weakness, headache, dizziness, erythromelalgia, and of course in case of thrombotic complications or hemorrhage. In the case of an increased platelet count, the first approach is to exclude reactive thrombocytosis secondary to infection, tissue damage, chronic inflammation, cancer, renal disorders, hemolytic anemia, blood loss, or postsplenectomy iron deficiency (ferritinemia). Other causes of primary thrombocytosis that must be excluded to fulfill diagnosis of ET are chronic myeloid leukemia, acute leukemia, myelodysplastic syndrome, and myeloid neoplasm.

The diagnosis of ET is then based on four major criteria: platelet count above 450 × 10^9/L, megakaryocyte proliferation with large and mature morphology, absence of other myeloid neoplasm and demonstration of JAK2V617F or other clonal marker, and no reactive thrombocytosis (Table 32.7). It should be noted that bone marrow (BM) examination in ET is often necessary to make an accurate morphologic diagnosis of ET and distinguish it from pre-fibrotic PMF or other myeloid neoplasms, with differences in the appearance of megakaryocytes. In the absence of JAK2V617F, the possibility of CML is addressed by screening for BCR-ABL1 mutation.

Table 32.7 The IPSET-thrombosis score

Risk factor	Hazard ratio	Score
Age > 60 years	1.50	1
Cardiovascular risk factors	1.56	1
Previous thrombosis	1.93	2
JAK2V617F	2.04	2

PMF is a rare disease, compared to PV or ET. Usually it can be discovered in the screening for anemia, a deterioration of the general status or painful and palpable splenomegaly. Hemogram revealed anomalies on the three cell lineages with the presence of nucleated red blood cells with teardrop-shaped erythrocytes, granulocytes precursors and megakaryocytes. However this myelophthisic smear is not specific and can be associated with myeloid malignancy, bone marrow fibrosis, or an infiltrating process. Thus, a BM biopsy with a careful morphologic evaluation and cytogenetic tests are necessary for the diagnosis of PMF.

The epidemiology of thrombosis in MPN has been recently reviewed [60]. Thrombotic events are more frequent in PV than in ET and PMF. Arterial thrombosis accounts for two-thirds of all thrombotic events. Large arteries (cerebral, coronary, and peripheral vessels) are frequently involved. In around 30% of patients with PV, and in 20% of patients with ET, the diagnosis is made after the occurrence of an initial thrombotic event, while during follow-up recurrence of thrombosis is observed in 20–33% of patients.

Other unusual sites of thrombotic occurrences, mainly Budd-Chiari syndrome and portal or mesenteric vein thrombosis, are also clinical features of MPN. Even in the absence of patent MPN, the search for "occult MPN" is mandatory as it is present in one-third of Budd-Chiari syndrome patients, as well as in nonmalignant, noncirrhotic, portal vein thrombosis patients [63]. In Budd-Chiari syndrome, the prevalence of MPN is 40.9% and the prevalence of JAK2V617F mutation is 41.1%; in portal vein thrombosis, the prevalence is 31.5% and 27.7% respectively. PV is more prevalent in Budd-Chiari syndrome than in portal vein thrombosis. These results validate the systematic screening for JAK2V617F mutation in the diagnostic workup of patients with splanchnic vein thrombosis and the notion of occult MPN. In a meta-analysis, JAK2 exon 12 and MPL515 mutations were extremely rare and, hence, should not be used when screening

splanchnic vein thrombosis patients for thrombophilias [64].

In the pathogenesis of MPN, several parameters must be taken into consideration: those clinical risk factors of the patient that are not specific to MPN and the disease-related risk factors [59, 60]. Age (>60 years old) and a history of thrombosis have consistently proven to be independent predictors of thrombosis in PV, ET, and PMF, with a 1.5 and 1.9 hazard ratio after a follow-up of 6 years, respectively [57]. Conventional risk factors for atherosclerosis (hypertension, hypercholesterolemia, diabetes, smoking) have also been associated with arterial thrombosis in MPN. Male gender is also associated with a higher risk of thrombosis, mainly myocardial infarction in patients with PV. In ET, male gender is also associated with a higher risk of venous thrombosis.

Concerning disease-related risk factors, mainly two parameters are taken into account: the JAK2V617F status and blood cell counts. JAK2V617F status could influence the risk of thrombosis in ET with a doubling of the risk and possibly also in PMF [60]. In PV, a higher JAK2V617F burden (>75% V617F allele) should allow to discriminate patients with a higher risk of thrombosis. Red cell count is a major cause of morbidity and mortality in PV and is a major determinant of blood viscosity and thrombosis. A hematocrit target below 45% is associated with a significant lower incidence of thrombosis and cardiovascular deaths. At variance, no study demonstrated a significant correlation between platelet count or function and thrombosis in PV and ET. Instead, extreme thrombocytosis seems to reduce the risk of thrombosis (maybe by an acquired von Willebrand disease) and is even associated with a significant increase risk of hemorrhage (almost tenfold higher above 1.000×10^9/L) [59–61]. Leukocytosis was also found to be to be an independent risk factor for arterial thrombosis in PV when white blood cell count exceeds 15×10^9/L [60]. In ET, leukocytosis was also an independent predictor of both thrombosis and survival.

Finally, by taking into account these risk factors, the IPSET (International Score of thrombosis in WHO—Essential Thrombocythemia) thrombosis score was developed (Table 32.7) [60, 61]. This simple score includes age, cardiovascular risk factors, previous thrombosis, and JAK2V617F and allows a prognostic model comprising three classes: low, intermediate, and high risk. Low-risk patients had a thrombosis-free survival of 1.03% patient-year, the intermediate-risk 2.35%, and the high-risk 3.56%.

Score: 0–1, low risk; 2, intermediate risk; and >3, high risk

Beside the identification and appropriate management of cardiovascular risk factors and the promotion of a healthy lifestyle, as in the general population, specific measures are recommended. Nevertheless, the supporting evidence is generally weak as there are only few prospective and interventional studies.

The efficacy of aspirin has been demonstrated in PV in a randomized trial, with a RR of 0.4 in the treated group compared to placebo for combined endpoint including nonfatal myocardial infarction, stroke, and major thromboembolism [65]. In ET the efficacy of aspirin has not been tested and is used to control clinical symptoms like erythromelalgia and transient ocular or neurologic episodes before cytoreduction. When platelet count is high, aspirin is not recommended due to the risk of bleeding.

PV patients should also be treated by phlebotomy to lower the hematocrit, and high-risk patients should also receive cytoreduction, mainly with hydroxyurea which is more effective in reducing thrombotic complications than phlebotomy alone. The goal of treatment is to achieve a target hematocrit <45%, as it has been demonstrated that, compared to a less intensive treatment with a hematocrit of 45–50%, the risk of thrombosis was decreased fourfold (2.7% versus 9.8% after a mean follow-up of 31 months) [66]. In ET, cytoreduction therapy is not indicated in patients without or with controlled cardiovascular risk factors; in high-risk patients, hydroxyurea and interferon-α are first-line therapy when cytoreduction is recommended [67]. New-generation drugs, with JAK2 inhibitory activity (ruxolitinib, lestaurtinib, pacritinib), have been found to be effective for the treatment of splenomegaly or

adult patients with myelofibrosis, but no data are available concerning their efficacy on thrombosis.

Impaired Endogenous Fibrinolysis or Hypofibrinolysis

Beside activation of coagulation, impaired endogenous fibrinolysis or hypofibrinolysis could also be associated with venous and/or arterial thrombosis. The association between fibrinolysis and clinical thrombosis is less documented for mainly two reasons: (1) assessment of endogenous fibrinolysis is much more complicated and less standardized than assessment of coagulation, and a test should ideally reflect the true physiological activity [68]; (2) genetic defect of fibrinolysis, such as homozygous plasminogen deficiency, is not associated with thrombosis. Similarly, a frequent polymorphism of PAI-1 (4G/5G) has been shown to be a potential risk factor for venous and arterial thrombosis, but due to conflicting results, this association is not really demonstrated and not useful in clinical practice [68].

We will try to briefly summarize the main data concerning hypofibrinolysis, recently reviewed [68, 69]. The clot lysis assay that measured the clot lysis time (CLT) is dependent of plasma levels of plasminogen, α2-antiplasmin, TAFI (thrombin-activatable fibrinolysis inhibitor), and PAI-1 (plasminogen activator inhibitor-1) [68, 69]. Briefly, the principle of this test is to add to citrated plasma a mix containing phospholipid vesicles, calcium chloride, tissue factor (to activate coagulation), and tPA (to initiate fibrinolysis). In a standard microplate reader in kinetic mode at 405 nm, the CLT is defined as the time between the midpoint of absorbance of the coagulation side of the curve and the midpoint of the lysis side of the curve [68]. This test does not really assess physiological fibrinolysis as it is performed with extrinsic t-PA, and it does not take into account the role of whole blood and the contribution of platelets and neutrophils. More recently, a global automated point-of-care fibrinolysis assay has been developed, but with only limited clinical data [69]. Contrary to thrombophilia, the assessment of each of the fibrinolytic pathway, by measuring, for example, levels of t-PA, PAI-1, or TAFI, is not contributory to assess endogenous fibrinolysis [69].

The association between hypofibrinolysis (defined as CLT above the 90th percentile) and a first episode of venous thromboembolic disease has been assessed, with OR comprised between 1.8 and 2.7, in the main four case-control studies [68, 70]. As for factor V Leiden or the prothrombin G20210A mutation, hypofibrinolysis is not associated with recurrent venous thrombosis nor with postthrombotic syndrome [68, 70]. Concerning the association of prolonged lysis time with acute coronary syndrome, it has been assessed in case-control as well as prospective studies with relative risk of recurrent events between 1.8 and 2.8 [68, 69]. In the largest prospective study, the PLATO trial (ticagrelor versus clopidogrel in patients with acute coronary syndromes), a 50% increase in CLT was associated with a 20% increase in cardiovascular death or all-cause deaths. According to these data, it seems that hypofibrinolysis, if confirmed, should be considered as a new marker of future coronary events [70]. On the other hand, its clinical interest in venous thrombosis remains limited.

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Part VII

Special Populations

Pediatrics

33

Amelia C. Lucisano, Christine M. Leeper, and Barbara A. Gaines

Introduction

Children are not simply little adults. This concept applies to many topics in pediatrics, including normal hemostasis, trauma-induced coagulopathy (TIC), and care of the bleeding pediatric trauma patient. Pediatric trauma patients are unique in their injury mechanisms, patterns, and pathophysiology. There is a significant body of literature which describes the differences between the pediatric and adult hemostatic systems. However, the consequences of these still-developing mechanisms for hemostasis and fibrinolysis after injury are not necessarily clear. Research has established that TIC occurs in pediatric patients as it does in adults, though a consensus definition of TIC, rates of occurrence, and implications of TIC in children remain areas of active research. Resuscitation strategies have been well studied in adults, resulting in well-defined goals for best practice, but a lack of research in children results in variable practice patterns across institutions. Special areas of concern in pediatric trauma include the high rate of traumatic brain injury and its association with TIC, physical child abuse and its unique impact on the trauma patient, and venous thromboembolism in high-risk pediatric patients.

Hemostasis in Children

The physiology of hemostasis in children after the age of 6 months is similar to that in adults. In neonates and infants, however, some key differences exist. The hemostatic and fibrinolytic systems rapidly develop during this early period. In premature infants, maturation of the system is likely somewhat accelerated, with studies showing it is roughly equivalent to term infants at 6 months of age. Various components of the hemostatic system exist in different quantities in infants compared to adults, with potential mechanisms for this phenomenon including differing consumption, synthesis, and clearance of proteins, along with differing enzymatic activity levels. Levels of many components reach normal adult ranges by 6 months of age, but some differences persist into early childhood. Traditional tests of coagulopathy, namely prothrombin time (PT) and activated partial thromboplastin time (aPTT, PTT) are often prolonged in the neonatal and infant period, due in part to a deficiency in vitamin K dependent clotting factors (including

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II, V, VII, IX, X, XI, and XII) seen in early life. Administration of vitamin K to newborns is, therefore, the standard of care to avoid hemorrhagic disease of the newborn. Differences also exist in inhibitors of the coagulation system, including lower levels of antithrombin III (ATIII) in infants compared to adults; however, other inhibitors (C1-esterase inhibitor and α2-macroglobulin) may exist in elevated levels and compensate at least partially for the deficiency in ATIII. Studies vary in their description of an infant's capacity for thrombin generation, but most suggest that it falls in the low-normal to normal range [1–7].

Another tradeoff may exist between infant platelets and levels of von Willebrand Factor (vWF). While the platelet count and volume are equivalent in neonates and infants compared to adults, platelet responsiveness is decreased in laboratory studies. Infant platelet dysfunction has been demonstrated in vitro by decreased granule secretion, decreased binding sites, and decreased aggregation. However, tests demonstrating the in vivo function show that the platelet function in an infant is largely preserved with bleeding times being normal to low in infancy. This may be due to the higher-than-adult levels and size of the circulating vWF in infants which contributes to enhanced platelet aggregation. The vWF levels may be elevated secondary to the birth process and/or an intrinsic processing difference at the cellular level [7–9].

The fibrinolytic capacity of young children may be impaired. Decreased levels of plasminogen, as well as impaired functionality of the protein, may exist in neonates and infants, contributing to impaired fibrinolytic capacity and tendency for thrombosis. Furthermore, the plasminogen activator inhibitor is at normal to elevated levels compared to adults and could exacerbate this blunting of fibrinolysis [3, 7, 10].

Overall, healthy neonates and children do not exhibit an increased tendency for spontaneous hemorrhage or thrombotic events. However, their coagulation systems are immature, and they compensate for decreased levels or impaired functionality of certain components by increased levels or overactivity of other components. It is hypothesized that small perturbations in this delicately balanced system may contribute to potential coagulopathy that is unique to very young children.

Pediatric Trauma-Induced Coagulopathy

As has been well documented in the adult population, severely injured children frequently manifest TIC, resulting in a tendency toward both hypo- and hypercoagulable states. This coagulopathy has been shown to be associated with poor outcomes including increased mortality, lengths of stay, transfusion requirements, and rates of organ failure. Though TIC can affect any patient who has suffered a traumatic insult, more severely injured patients as well as those with particular injury patterns, particularly traumatic brain injury, are known to be at highest risk [11–18]. The following discussion will detail different ways of defining TIC in children based on different laboratory tests, including both conventional coagulation tests (CCTs) and viscoelastic hemostatic assays (VHAs).

Conventional Coagulation Test-Based Studies

Many studies of TIC in the pediatric population utilize abnormalities in conventional coagulation tests, (PT, INR, PTT, fibrinogen, and platelet count) to define TIC. However, lack of a uniform definition of TIC by laboratory values makes the comparison between these studies difficult [19]. A large systematic review from 2014 found that the incidence of TIC among the mostly retrospective studies ranged from 10% to 77% [12]. Furthermore, a majority of these studies were conducted in TBI patients, who are known to be at a higher risk for TIC, with only a few being conducted in a general trauma population. Of studies conducted in critically-ill polytrauma patients, the range of patients with TIC narrowed to 27–38% in four studies; a fifth found that 72% of all patients needing a transfusion had an

abnormal PT. All five of these studies found an association between TIC and mortality [17, 20–23]. Regarding the definition of TIC, one group sought to better define TIC by establishing an appropriate cutoff for an elevated INR. They found that an INR of 1.3 or greater captured a clinically significant mortality rate and had the greatest sensitivity and specificity for mortality among a general critically ill pediatric trauma population. While elevated INR at admission and 24 h was predictive of mortality, increased product transfusion was not associated with improved INR over time, suggesting that elevated INR alone may not be an ideal target to guide resuscitation [20].

Viscoelastic Hemostatic Assay-Based Studies

Viscoelastic hemostatic assays (VHAs) including thromboelastography (TEG, Haemonetics, Braintree, MA) and thromboelastometry (ROTEM, Instrumentation Laboratory, Bedford, MA) are functional tests that use whole blood to measure various aspects of clot formation, stability, and degradation. The results of the test develop in real time, compared to CCTs, and they provide more granular data about the status of the patient's hemostatic state which can be used to drive resuscitation measures [24–26]. In adult trauma patients, TEG shows correlation with CCTs and accurately predicts the need for blood product transfusion as well as massive transfusion [26] and has shown superiority compared to five standard CCTs, particularly in patients with head injury, hemorrhagic shock, and those requiring a transfusion [27]. Furthermore, VHAs capture data regarding fibrinolysis which is not reflected in the traditional tests of coagulopathy [19]. A study of the use of TEG in adult trauma patients to detect abnormal fibrinolysis found that most trauma patients arrive with an abnormal LY30 (percent decrease in maximum amplitude (MA) at 30 min compared to time zero): 18% of the population arrived in a state of hyperfibrinolysis (LY30 $\geq$ 3%) and 46% arrived in a state of fibrinolysis shutdown (LY30 $\leq$ 0.08%). Both abnormal states were associated with higher mortality compared to the physiologic state [28]. These reasons, in combination with the above mentioned finding that INR is not a reliable target for resuscitation [20], make VHAs an ideal method for assessing an injured child for coagulopathy.

An early study on the use of TEG in pediatric trauma patients found that activated clotting time (ACT), k-time, and α-angle correlated with PTT and MA correlated with platelet count. All of these values independently predicted the need for packed red blood cells (PRBCs) and plasma transfusion within 6 h [18]. TEG has subsequently been utilized to characterize fibrinolysis phenotypes after injury in children. Leeper et al. found that among a cohort of pediatric trauma patients, 19.6% had hyperfibrinolysis on TEG at admission, while 38.3% had shutdown physiology. Both abnormalities correlated with mortality and need for blood transfusion, while shutdown was a particularly poor sign as it also correlated with disability and deep vein thrombosis (DVT) [29]. Children in this study showed equivalent rates of hyperfibrinolysis compared to the previously described study of adult trauma patients; however, they demonstrated a normal fibrinolytic pattern slightly more frequently and were less likely to show evidence of the shutdown physiology at the time of admission (38.3% v. 46%) [28, 29]. Possible explanations for these differences between adult and pediatric trauma patients could include variation in the timing of tests, differences in the degree of tissue damage, and the preponderance of traumatic brain injury in injured children, as well as perhaps a superior compensatory response to preserve normal fibrinolysis in children, compared to that in adults.

Liras et al. similarly concluded that hyperfibrinolysis on admission TEG was an independent predictor of mortality in pediatric patients [30]. In a study evaluating changes in fibrinolysis over time, Leeper et al. found that remaining in or trending toward fibrinolysis shutdown in the days after injury was associated with worse clinical outcomes including death, disability, and DVT [31]. These findings are concordant with adult studies investigating sustained fibrinolysis

shutdown [28, 32, 33]. Leeper et al. also found that the predominant fibrinolytic phenotype among pediatric patients early after injury (<1 h) was that of normal fibrinolytic physiology. This was followed by a trend toward shutdown physiology in the hours to follow. Perhaps a manifestation of children's superior compensatory mechanisms, this finding may represent an early window of opportunity in injured children to mitigate the coagulopathy that is to follow [34].

Despite the growing body of literature supporting the use of VHAs for the assessment and treatment of the pediatric trauma patient, one survey of providers caring for pediatric trauma patients found that only 31% of respondents use VHAs regularly, despite the tests being available to 63% of those surveyed [35]. Effort should be made to implement protocols which utilize VHAs in the care of the pediatric trauma patients, and the results of these programs should be studied further.

Resuscitation of Pediatric Trauma Patients

Pediatric-specific resuscitation protocols are necessary given the baseline physiologic differences between adults and children. Infants and children, particularly those less than 50 kg, have higher circulating blood volumes by body weight compared to adults. An infant has a blood volume of 90 mL/kg, while a young child has a blood volume of 70 ml/kg [36]. Therefore, estimation of the blood volume, volume loss, and dosing of blood products must be weight based [37]. Another key feature of the pediatric population is their substantial physiologic reserve, and therefore signs or symptoms of blood loss may not be apparent as early as in adults or if present may be a sign of impending circulatory collapse [36, 38]. One study of hypotension among a large cohort of pediatric trauma patients found it to be an extremely poor prognostic indicator; patients who were hypotensive on arrival to the emergency department were nearly 13 times more likely to die compared to normotensive patients [39]. Early recognition of hemorrhagic shock, coagulopathy, and need for transfusion are of vital importance. However, given the robust physiologic reserve of pediatric patients and the divergence of normal vital signs from those of adults with decreasing age, identification of pediatric patients with hemorrhagic shock and need for transfusion can be particularly challenging.

Massive Transfusion Protocols and Balanced Resuscitation

Trauma patients often show sustained substantial blood loss prior to presentation to definitive care or have a high likelihood of ongoing blood loss. TIC can contribute to the ongoing blood loss and resuscitation itself can exacerbate coagulopathy. Massive transfusion (MT) protocols were developed for adult patients in order to streamline the delivery of large volumes of blood products to patients with the most need. The Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial and other studies have shown that MT protocols in adults decrease the mortality, coagulopathy, and other complications, primarily through the successful delivery of balanced ratios of products, ideally in a 1:1:1 fashion for PRBCs, plasma, and platelets [40–43].

Adult MT protocols are not directly applicable to pediatric patients. As discussed previously, component therapy in a MT protocol must be dosed in a weight-based format. Table 33.1 details pediatric weight-based dosing for the different components to be used in a MT protocol [44, 45]. Pediatric MT occurs less frequently

Table 33.1 Dosing of components for pediatric massive transfusion (patient weight <50 kg)

Product	Dose
Crystalloid	10 mL/kg
Packed red blood cells (PRBCs)	10–20 mL/kg
Plasma	10–20 mL/kg
Platelets	10 mL/kg
Tranexamic acid (TXA)	15 mg/kg (max dose 1 g)

mL/kg milliliters per kilogram, *mg/kg* milligrams per kilogram

than in adults, with one large study of patients in the National Trauma Data Bank (NTDB) finding that only 0.04% of pediatric patients received an MT [46]. Regarding balanced resuscitation in pediatric patients, several studies failed to prove the same benefit as has been demonstrated in adults [47–49]; importantly, none of these studies actually achieved a 1:1:1 ratio of products in their MTP cohorts. This balanced ratio is difficult to achieve in children given their smaller circulating blood volumes. However, two studies demonstrate an association between higher ratios of plasma:PRBCs and mortality [50, 51] and one study showed that a ratio of 1:1 for PRBCs:plasma was associated with the highest survival, while ratios involving higher proportion of PRBCs were associated with lower survival [52]. Despite the variation among the evidence in children, balanced resuscitation remains a goal in the pediatric trauma population [53].

Algorithms and scoring systems predicting the need for massive transfusion in trauma patients are particularly useful in guiding clinical care. In adults, the American College of Surgeons (ACS) Trauma Quality Improvement Program (TQIP) advises the activation of a MT protocol when two out of the four criteria are met: Assessment of Blood Consumption (ABC) score (one point each for pulse >120, SBP <90, positive Focused Assessment with Sonography in Trauma (FAST), or penetrating injury) of two or more, persistent hemodynamic instability, active bleeding requiring emergent intervention, blood transfusion in the trauma bay [54]. In adults, an ABC score of two or greater was 75% sensitive and 86% specific for predicting the need for MT [55]. Unfortunately, this scoring system does not perform as robustly in a pediatric population. A study of the ABC score as described above found that in pediatric patients the sensitivity of the ABC score for predicting the need for MT dropped to 29% [56]. Shock index (SI) (maximum heart rate divided by minimum systolic blood pressure) has been studied in adult trauma patients and when compared to the ABC score, it has a slightly higher sensitivity for predicting the need for massive transfusion and is also technically easier to calculate [57]. In pediatric patients, the SI can be pediatric-adjusted (SIPA), to more accurately reflect the pediatric age-adjusted normal vital signs: the cutoffs for normal SIPA include <1.22 (age 4–6), <1.0 (age 7–12), and <0.9 (age 13 and above). The SIPA showed an improved discriminatory capacity compared to SI for several poor outcomes including ISS, need for transfusion within 24 h, and mortality among a pediatric population [58]. When the traditional ABC score is modified to include SIPA in place of pulse and SBP (ABC-S), the sensitivity of the score for predicting the need for MT is modestly improved to 65% [56]. The score was further modified to form the ABCD score, incorporating both base deficit and lactate, which demonstrated the highest discriminatory capacity for MT with a sensitivity of 77.4% and specificity of 78.8% [59]. It should be noted that the authors of many of these studies comment on the fact that the ABC score may be inherently limited in the pediatric population given the low rate of penetrating injury and positive FAST exams.

Finally, there is considerable variability in the definition of pediatric MT by institution, the most common modern widely-accepted definition of pediatric MT is >40 mL/kg total blood product transfused in 24 h; however, this is limited in that it is calculated retrospectively and has utility mainly in research definitions. No consensus exists for the triggers for pediatric MT and the most commonly cited indication in the literature is physician discretion [60].

VHA-Based Resuscitation

As discussed previously, VHAs have been shown to correlate with CCTs and certain findings predict mortality and poor outcomes. The results from these assays are available faster than the traditional tests, and they can guide specific steps in the resuscitation algorithm including the administration of plasma, platelets, PRBCs, cryoprecipitate, and tranexamic acid (TXA). The ACS TQIP publishes guidelines for transfusion, including TEG-based transfusion triggers, from which our institution has derived the following protocol as listed in Table 33.2 [54].

Table 33.2 Example of TEG-based transfusion algorithm used at the American College of Surgeons (ACS) Level 1 Pediatric Trauma Center

TEG parameter	Normal range	Transfusion trigger	Product	Dose
ACT	86–118 s	>128 s	Plasma	20 mL/kg
α angle	64–80°	<60°	Cryo	1 unit/10 kg
K value	0–2.5 min	>2.5 min	Cryo	1 unit/10 kg
MA	52–71 mm	<55 mm	Platelets	15 mL/kg
LY30	0–8%	>3%	TXA	> 12 yo (adult dose): 1 gm loading dose over 10 min, followed by 1 gm infusion over 8 h ≤12 yo: 15 mg/kg (max dose 1 gm) loading dose over 10 min, followed by 2 mg/kg/hr infusion over 8 h

ACT Activated clotting time, *sec* seconds, *mL/kg* milliliters per kilogram, *Cryo* Cryoprecipitate, *kg* kilogram, *min* minute, *MA* Maximum amplitude, *mm* millimeter, percent decrease in MA at 30 min compared to time 0 (LY30), *yo* years old, *gm* gram, *h* hours

Whole Blood Transfusion

Transfusion of cold-stored low titer group O whole blood (LTOWB) allows the trauma patient to receive RBCs, plasma, and platelets in a physiologic ratio, eliminating the increased volume and time needed to transfuse these three products in the traditional fashion. Multiple studies of WB transfusion in an adult trauma patient population achieved higher ratios of plasma:RBCs and platelet:RBCs in WB groups compared to those receiving conventional component-based therapy, without any adverse transfusion-related reactions [61–63]. The first study of WB use in a pediatric trauma population found it to be safe, with no evidence of adverse transfusion-related reactions and no difference in biochemical markers of hemolysis in group-O and non-group-O patients. Furthermore, the WB was delivered significantly faster compared to the administration of a corresponding unit of RBCs, plasma, and platelets in a historical cohort [64]. In another study of WB in children from the same group, the platelet count and function in pediatric trauma patients who received cold-stored WB was no different from a cohort of trauma patients who had received traditional room temperature component platelets [65]. This group now utilizes WB in their pediatric MT protocols for children older than one year of age, as depicted in Fig. 33.1. LTOWB is a safe, efficient, and the ideal initial resuscitative fluid for the bleeding pediatric trauma patient.

Tranexamic Acid (TXA)

TXA is an anti-fibrinolytic drug with proven effectiveness in adult trauma patients for reducing mortality secondary to bleeding, as demonstrated in the Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage 2 (CRASH-2) Trial [66]. The CRASH-3 trial was subsequently published, and it showed that mild to moderate TBI patients who received TXA also had a lower mortality [67]. Neither study population included children, and therefore the safety and effectiveness of TXA use in pediatric trauma patients requires further investigation. Two retrospective studies examining TXA use in pediatric trauma patients arrive at discordant conclusions. One study in pediatric patients in a combat setting with severe abdominal or extremity trauma found that those who received TXA had decreased mortality without an increase in thromboembolic complications [68]. Another study of a large database of Japanese pediatric trauma patients, which used propensity matching, found that the TXA group had a higher rate of seizures compared to the group that did not receive TXA, but the two groups had equivalent rates of thromboembolic complications and mortality [69]. Despite conflicting evidence among these two studies of pediatric trauma patients, multiple studies of the use of TXA in children undergoing non-traumatic surgical procedures exist and suggest that TXA is safe and may be associated with

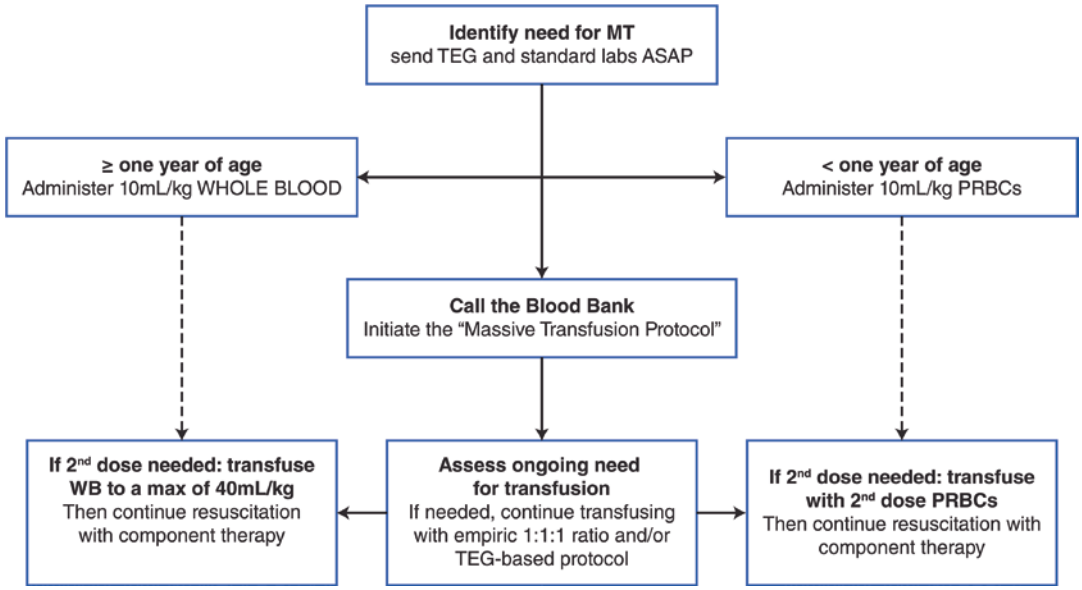


Fig. 33.1 Example of the pediatric MT protocol utilizing WB used at the American College of Surgeons (ACS) Level 1 Pediatric Trauma Center

lower transfusion requirements and/or blood loss [70–72]. The Traumatic Injury Clinical Trial Evaluating Tranexamic Acid in Children (TIC-TOC) trial, a randomized controlled trial of two doses of TXA and placebo, currently in the pilot phase of enrollment, will ideally eventually provide robust evidence regarding the safety and effectiveness of empiric TXA in the treatment of the bleeding pediatric trauma patient [73].

Disorders of fibrinolysis exist on a spectrum, with a majority of trauma patients arriving to the hospital in a state of abnormal fibrinolysis [33]. Those in a state of hyperfibrinolysis (LY30 ≥ 3% on TEG) may benefit from anti-fibrinolytic therapy and in this way TEG could be used to guide resuscitation efforts. However, pediatric patients in hyperfibrinolysis have been shown to correct on their own without the use of TXA, as demonstrated by all the individuals with hyperfibrinolysis in one cohort of pediatric trauma patients [31]. Patients with physiologic levels of fibrinolysis or fibrinolysis shutdown may not benefit from TXA administration, and theoretical harm may result from administering anti-fibrinolytic agents to patients in a potentially prothrombotic state. As discussed above, anti-fibrinolytic therapy in children needs investigation to determine the safety, efficacy, timing, and indications for its administration.

Special Considerations in the Pediatric Population

Traumatic Brain Injury

TBI is a known risk factor for the development of TIC and is a predominant cause of morbidity and mortality in children [74–78]. Potential mechanisms for the induction of coagulopathy after brain injury are numerous. TIC has been detected early even after isolated TBI, suggesting that the release of brain-derived substances via disruption of the blood–brain barrier may play a role in the development of coagulopathy [79, 80]. Hypoperfusion leading to activation of the protein C pathway is suspected to play a role in the development of TIC after TBI [77, 81]. Platelet dysfunction has been well described after TBI [80, 82, 83]. One study in adult patients revealed that isolated TBI patients display a unique coagulopathy on TEG, characterized by impaired clot

formation rather than abnormal fibrinolysis [84]. More research is needed to fully understand the mechanism behind TIC in the setting of TBI, with the hope of developing interventions to mitigate the particularly poor outcomes for this injured subgroup.

Pediatric TBI patients have been shown to manifest coagulopathy at a particularly high rate, with one study describing 42.8% of TBI patients developing coagulopathy, with an increasing incidence with increasing head AIS [76]. Severe TBI in a pediatric population has been shown to be associated with a trend toward or remaining in fibrinolysis shutdown, a poor prognostic indicator [85]. Patients with severe TBI also had an increased rate of shutdown physiology compared to non-TBI patients, detectable as soon as 1 h after injury [34].

Research in adults suggests that early resuscitation with the plasma can be particularly beneficial to TBI patients. Sperry et al. conducted the Prehospital Plasma during Air Medical Transport in Trauma (PAMPer) Trial, a randomized trial of pre-hospital plasma versus standard of care in adult trauma patients, and found that pre-hospital plasma administration was linked to a decreased mortality at 30 days, with a significant benefit observed in the TBI subgroup [86]. Another study of adult trauma patients, compared to those who received early plasma compared to PRBCs, showed that the plasma group had improved neurologic and functional outcomes [87]. No such studies have been conducted with pediatric trauma patients; however, it has been shown that pediatric TBI patients who received delayed plasma administration or over-resuscitation with plasma had higher incidence of fibrinolysis shutdown. Furthermore, in those with severe TBI and plasma transfusion, the rates of fibrinolysis shutdown and morbidity and mortality were exceedingly high [85]. In children with TBI there may exist a delicate balance between beneficial early resuscitation with plasma and over-resuscitation which worsens TIC.

Also of critical importance for TBI patients is the prevention and treatment of early hypotension. Hypotension, even in short episodes, is well known to be associated with poor outcomes in TBI patients including a significantly increased risk of mortality [88, 89]. Multiple studies show that the threshold for defining hypotension in TBI patients should be higher than the standard definition for hypotension [90, 91] and this same finding was demonstrated in pediatric trauma patients, based on the strong link between early hypotension and mortality in the setting of TBI [92].

Child Abuse

Child abuse is a public health epidemic that inflicts both immediate and lasting physical and psychosocial consequences upon its victims. The most common cause of long-term morbidity and mortality secondary to child abuse is abusive head trauma (AHT) [93, 94]. AHT disproportionately affects young children and is the leading cause of head injury in the less than one-year-old population. These children have particularly poor outcomes; AHT has a case-fatality rate that likely exceeds 20% and a majority of survivors are left with permanent disability [94–96]. AHT patients frequently have hypoxic-ischemic changes in their brain, thought to be in part related directly to their brain injury but also in part as a consequence of hypoxia that may occur secondary to apnea or disordered breathing from trauma to the spine or brainstem inflicted during the abusive action [97–101]. As with TBI in general, hypoxic-ischemic injury to the brain may contribute to the development of TIC. Not surprisingly, pediatric AHT patients manifest TIC frequently, with one study finding 35% of an AHT cohort with an INR of 1.3 or greater. TIC as defined by INR was the strongest predictor of mortality, increasing the odds of death by 3.65 times or more [102]. Another study of TEG in AHT compared to accidental head trauma patients found that AHT patients were more likely to have an elevated LY30 on admission and patterns on TEG which indicated abnormal clot strength correlated with mortality [103]. AHT patients clearly represent a group at risk for a potentially unique TIC.

Venous Thromboembolism in Children

TIC may manifest as a hypercoagulable state, which can lead to venous thromboembolism (VTE) events. Overall, VTE occurs significantly less frequently in children compared to adults, but the risk can be substantial in high-risk groups [104]. Studies vary widely in their reported rates of VTE among pediatric trauma patients, ranging from <1 to 10% [105–108]; however, higher risk groups may have rates in the 10–25% range [106, 109]. The following risk factors for VTE in pediatric trauma patients have been identified: increasing age, injury severity, indwelling central venous catheters, total parenteral nutrition, immobility, pressor support, TBI, and AHT [105, 107, 110–112]. The primary clinical consequence of VTE, aside from significant additional costs related to the VTE treatment, is post-thrombotic syndrome (PTS), which results in painful swelling of the affected extremity with skin changes including dilated veins, dermatitis, and venous stasis ulcers. Though there is a significant variation on the reported rates of PTS in the literature, most studies report that the rates of pediatric PTS are likely similar to those observed in adults, in the 15–45% range [105, 113–115].

Unfortunately, the highest risk groups, including those with TBI and significant trauma including solid organ injury, may be unable to receive VTE prophylaxis due to a prohibitively high bleeding risk. Furthermore, though the effectiveness of pharmacological VTE prophylaxis is assumed to be similar for post-pubertal adolescents to that in adults, the efficacy of these medications in children is largely unknown due to the lack of pediatric clinical trials. Given the unclear efficacy of medications used for VTE prophylaxis in children, there exists significant uncertainty regarding which children should subsequently be given prophylaxis.

The Eastern Association for the Surgery of Trauma (EAST) published guidelines in 2016 that suggest the use of pharmacological prophylaxis in children: (1) age 15 and older with low bleeding risk and (2) younger than 15 but postpubertal with an ISS >25. The group recommended against prophylaxis in prepubescent children [116]. Further research is needed on the safety and efficacy of pharmacologic prophylaxis in children to guide its use in younger children who are at an increased risk for VTE due to the presence of one or more of the above risk factors.

Summary

TIC occurs in a significant number of severely injured pediatric trauma patients. High-risk groups include those with traumatic brain injury and physical child abuse. Differences in the developing hemostatic system in children may contribute to a tendency toward coagulopathy. Topics of critical importance for ongoing research include resuscitation strategies and prevention and management of VTE in pediatric trauma patients.

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Traumatic Brain Injury-Induced Coagulopathy

34

Jing-fei Dong, Fangyi Zhang, and Jianning Zhang

Introduction

Trauma is the leading cause of death among people aged 50 and younger, and it is a major cause of death for all adults, contributing substantially to health care costs and lost productivity. Uncontrolled hemorrhage accounts for 30–50% of all trauma fatalities [1, 2] and is caused by direct injury to the vasculature and secondary coagulopathy. Trauma-induced coagulopathy can occur minutes after injury in a prehospital setting and progress rapidly, significantly increasing the risk of secondary bleeding, disability, and death [3, 4]. A vicious cycle of coagulopathy, hypothermia, and metabolic acidosis is often referred to as a lethal triad for patients with severe trauma [5, 6]. Clinical and laboratory studies have consistently shown that coagulopathy is also common in patients with traumatic brain injury (TBI).

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Epidemiology and Risk Factors

The prevalence of TBI-induced coagulopathy (TBI-IC) varies considerably in individual reports, ranging from 10% to 97.2% [7–9]. Several factors contribute to this large variation. First, TBI is a collective term for several types of injuries. Coagulopathy is more common in patients with penetrating TBI than in those with blunt injury [9–11]. Patients with cerebral parenchymal injuries (e.g., contusion) are more likely to develop TBI-IC than those with brain structural compressing injuries such as epidural hematoma. Second, TBI-IC is diagnosed by different and nonstandardized laboratory tests (Table 34.1) [7–9]. These tests and the timing with which they are performed define abnormalities in different aspects of the hemostasis system. Third, TBI-IC may occur in more than 80% of patients with severe TBI, as defined by clinical injury severity scores such as the Glasgow Coma Scale (GCS) or the Abbreviated Injury Scale (AIS) head [12], but it is found in less than 1% of patients with mild TBI [13]. A meta-analysis of 34 studies of civilian TBI published from 1966 to 2007 revealed an overall TBI-IC prevalence of 32.7% [9]. A systematic review conducted 5 years later of 22 studies found that the pooled proportion of isolated TBI with coagulopathy was 35.2% (95% CI: 29.0–41.4) [14]. This significant epidemiological variation in clinical studies illustrates the inconsistency in defining TBI-IC, the large gap

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Table 34.1 Laboratory tests used to define TBI-IC

Test	Mechanism	Pathway
Individual test		
PT/INR[a]	Time for plasma to clot	Extrinsic and common pathway
INR[a]	The ratio of PT-test/PT-normal	Extrinsic and common pathway
PTT/aPTT[a]	Time for plasma to clot	Intrinsic and common pathway
TFPI	Inhibitor of FXa	Common pathway
D-dimer	Fragments of fibrin cleaved by plasmin	Fibrinolysis
PAI-1	Inhibitor of tPA and uPA	Fibrinolysis
α2 antiplasmin	Inhibitor of plasmin	Fibrinolysis
Fibrinogen	Platelet aggregation and fibrin clot	Platelet hemostasis and clot formation
Platelet counts[a]	Platelet activation and aggregation	Platelet hemostasis
Global test		
TEG[a]	A computerized device to measure clot formation and fibrinolysis under venous flow	Clot formation/strength and fibrinolysis
ROTEM[a]	A different version of TEG	
DIC score	A combination of platelet count, fibrinogen/fibrin, and PT	Primary hemostasis

PT prothrombin time, *INR* international normalized ratio, *PTT/aPTT* partial thromboplastin time/activated partial thromboplastin time, *TFPI* tissue factor pathway inhibitor, *FXa* activated factor X, *PAI-1* plasminogen activator inhibitor-1, *tPA* tissue plasminogen activator, *uPA* urokinase (or urokinase-type plasminogen activator), *TGE* thromboelastography, *ROTEM* rotational thromboelastometry

[a]Most common tests for TBI-IC

between laboratory-defined and clinically presented coagulopathy, and the need for standardized diagnosis and risk-stratification strategies.

In a retrospective study of 3114 patients with isolated TBI recorded in the Trauma Registry of the German Society for Trauma Surgery (TR-DGU), multivariate analysis identified the following as independent risk factors for the development of acute TBI-IC: (1) severe injury defined by either AIS head ≥3 or GCS ≤8 at the scene; (2) hypotension at the scene or upon arrival at the emergency room (systolic blood pressure ≤90 mmHg); (3) prehospital administration of intravenous fluids of ≥2000 ml; and (4) age ≥75 years [15]. Low hemoglobin, penetrating cerebral injury, evidence of cistern involvement, midline shift, cerebral edema, and subarachnoid hemorrhage detected by computerized tomography (CT) or magnetic resonance imaging (MRI) scans have also been associated with TBI-IC in smaller studies [16–18]. There are no published epidemiological data on the impact of race and sex on the development of TBI-IC and associated outcomes.

TBI-IC develops early and progresses rapidly upon injury [19]. Lustenberger et al. reported that coagulopathy defined by thrombocytopenia, elevated international normalized ratio (INR, which is calculated from the prothrombin time [PT]), or prolonged activated partial thromboplastin time (aPTT or PTT) occurred 23 ± 2 hours after admission to the emergency room, with a mean duration of 68 ± 7.4 hours [20]. Coagulopathy develops significantly faster in patients with severe or penetrating TBI.

Pathogenesis

Common causes of coagulopathy after trauma to the body and limbs induce significant blood loss (hemorrhagic shock), hemodilution, and hypothermia due to fluid resuscitation, systemic metabolic acidosis due to substantial tissue ischemia, dysfunctional platelets and coagulation, and hyperfibrinolysis [21–25]. However, coagulopathy is equally common in patients with isolated TBI [7, 9, 26]. This high prevalence occurs in TBI trauma patients despite a lack of significant blood loss, limited fluid resuscitation, normothermia (often febrile), and lack of systemic metabolic acidosis [7–9]. These differences in causality suggest that TBI-IC is mechanistically distinct from deficient and dilutional coagulopathy arising after extracranial trauma and hemorrhagic shock. A critical question is how a

localized brain injury is rapidly disseminated to alter the systemic hemostasis system, which is not directly affected as in patients with extracranial trauma and hemorrhagic shock. Brain-derived extracellular vesicles (BDEVs) have emerged as a key disseminating and causal factor for TBI-IC.

The human primary hemostasis system is composed of four distinct but interdependent components (Fig. 34.1). Hemostasis is initiated when platelets adhere to the subendothelium exposed at the site of vascular injury and become activated and aggregated to seal the wound. This platelet plug is rapidly stabilized by cross-linked fibrin derived from fibrinogen cleaved by thrombin [27, 28], which is generated through the extrinsic and intrinsic pathways of coagulation. The extrinsic pathway is initiated by tissue factor (TF) and is drastically enhanced and accelerated by anionic phospholipids, such as phosphatidylserine (PS) and phosphatidylethanolamine. The expression of anionic phospholipids is ubiquitous in all cells. However, anionic phospholipids such as PS normally reside on the inner leaflet of

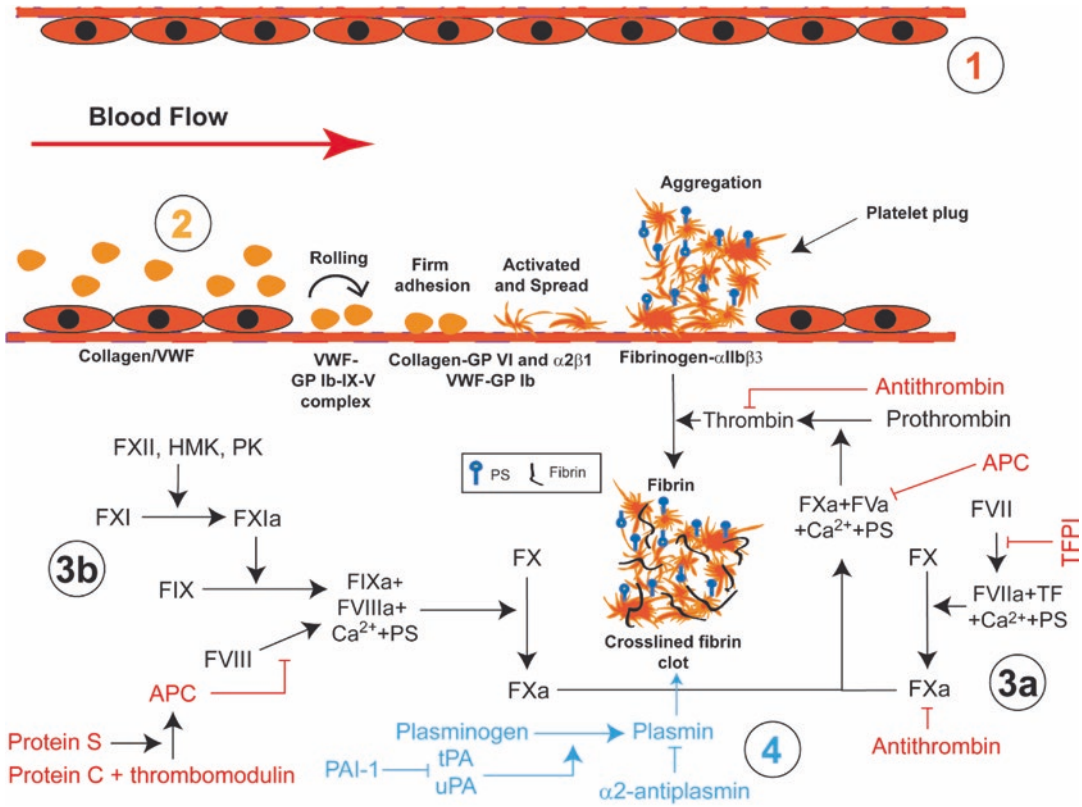


Fig. 34.1 Schematic illustration of the human hemostasis system, which consists of four closely interlinked, but distinct components. First is the intact endothelium (dot with red 1), which is anticoagulant and platelet- and leukocyte-repellent. Second is platelets and adhesive ligands (dot with orange 2). Upon vascular injury, the subendothelium is exposed. Platelets are tethered to VWF in the sudendothelium through the GP Ib-IX-V complex and engaged with the subendothelial collagen through the integrin α2β1/GP VI and become activated. Activated platelets aggregate by fibrinogen crosslinking and express anionic phospholipids such as PS. Third is coagulation through extrinsic (tissue factor, dot with black 3a) and intrinsic (contact, dot with black 3b) pathways. PS exposed on the surface of activated platelets interacts with tissue factor to activate FVII to FVIIa, which activates FX. The activated FXa promotes the generation of thrombin to cleave fibrinogen into fibrin, which is then polymerized rapidly (black arrows indicate catalytic interactions and red lines indicate inhibitory factors). Fourth is fibrinolysis, which is triggered by the release of tPA and uPA, which catalytically activates plasminogen to plasmin to dissolve fibrin polymers and re-establish blood flow

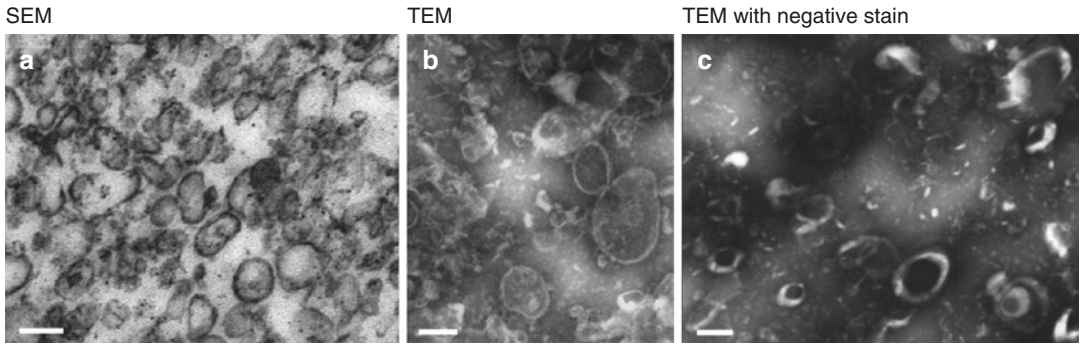


Fig. 34.2 Electron microscopy images of extracellular vesicles found in the peripheral blood of mice subjected to severe lateral percussion injury. Cell-free plasma from TBI mice was centrifuged at 100,000×g for 60 minutes at 4 °C (twice) and extracellular vesicles were suspended in PBS, fixed first with 2.5% glutaraldehyde (24 hours at 4 °C) and then with 1% osmium tetroxide (1.5 hours at 4 °C), and processed for electron microscopy (EM). (**a**) Scan EM of concentrated extracellular vesicles ranging from 50 to 350 nm (bar = 200 nm). (**b**) Transmission EM of extracellular vesicles showing high-density membrane structure (bar = 200 nm), suggesting that these vesicles form granuale-like structures with embedded residual cytoplasma and other cargo contents. (**c**) The membrane structure of extracellular vesicles detected by TEM is greatly enhanced by negative stain with phosphotungstic acid (TEM, bar = 200 nm)

the platelet membrane bilayer [29], whereas neutral phospholipids (e.g., phosphatidylcholine) reside on the external leaflet [30]. This asymmetric distribution of membrane phospholipids is maintained by active transporters [31, 32] but quickly lost on the activated platelets aggregated at the site of vascular injury, thus exposing PS to initiate and propagate coagulation on the surface of activated platelets [29–31]. Unlike anionic phospholipids, there is a very limited amount of TF in the circulation in a resting state because (1) platelets express no or very little TF [33, 34]; (2) monocytes [35–38] and endothelial cells [39, 40] express TF only after induction by inflammatory and ischemic stimuli; and (3) plasma contains very low levels of soluble TF (1–10 ng/mL [41–43]), which appears to be encrypted or otherwise insufficient to trigger coagulation. By contrast, TF is abundantly expressed on cells in the vessel wall, such as fibroblasts and smooth muscle cells, and on nonvascular cells such as astrocytes, epidermal cells, and renal glomeruli. This selective expression prevents spontaneous intravascular coagulation during homeostasis of the body while ensuring the availability of TF at the site of vascular injury for injury-induced hemostasis. A long-standing question is how TF from these nonblood cells is integrated into activated platelets at the site of vascular injury to initiate coagulation. Extracellular vehicles (EVs) including BDEVs may again play a critical role by providing TF to platelets through membrane fusion [44].

EVs are ~1 μm cellular vesicles (Fig. 34.2) [45] that include membrane fragments, intracellular organelles, exosomes, and associated cargo molecules from cells undergoing either active microvesiculation [46, 47] after the membrane skeleton is disrupted [48–50] or apoptosis [51, 52]. Membrane EVs express procoagulant anionic phospholipids on their surface [53, 54]. The brain is highly sensitive to ischemic and traumatic insults causing it to release BDEVs [53, 55–57]. These BDEVs are more active in triggering coagulation than EVs from platelets or other cells because they express not only TF but also significantly higher levels of PS on their surface [55]. Phospholipids account for ~25% of the dry weight of an adult brain, but only ~10% of the dry weight of any other tissues or organs [58–61]. Upon being released into circulation, these PS- and TF-exposing BDEVs could serve as circulating mini-platforms, on which coagulation is initiated and propagated in fluid phase of blood instead of the site of vascular injury (Fig. 34.3). BDEVs could also activate platelets

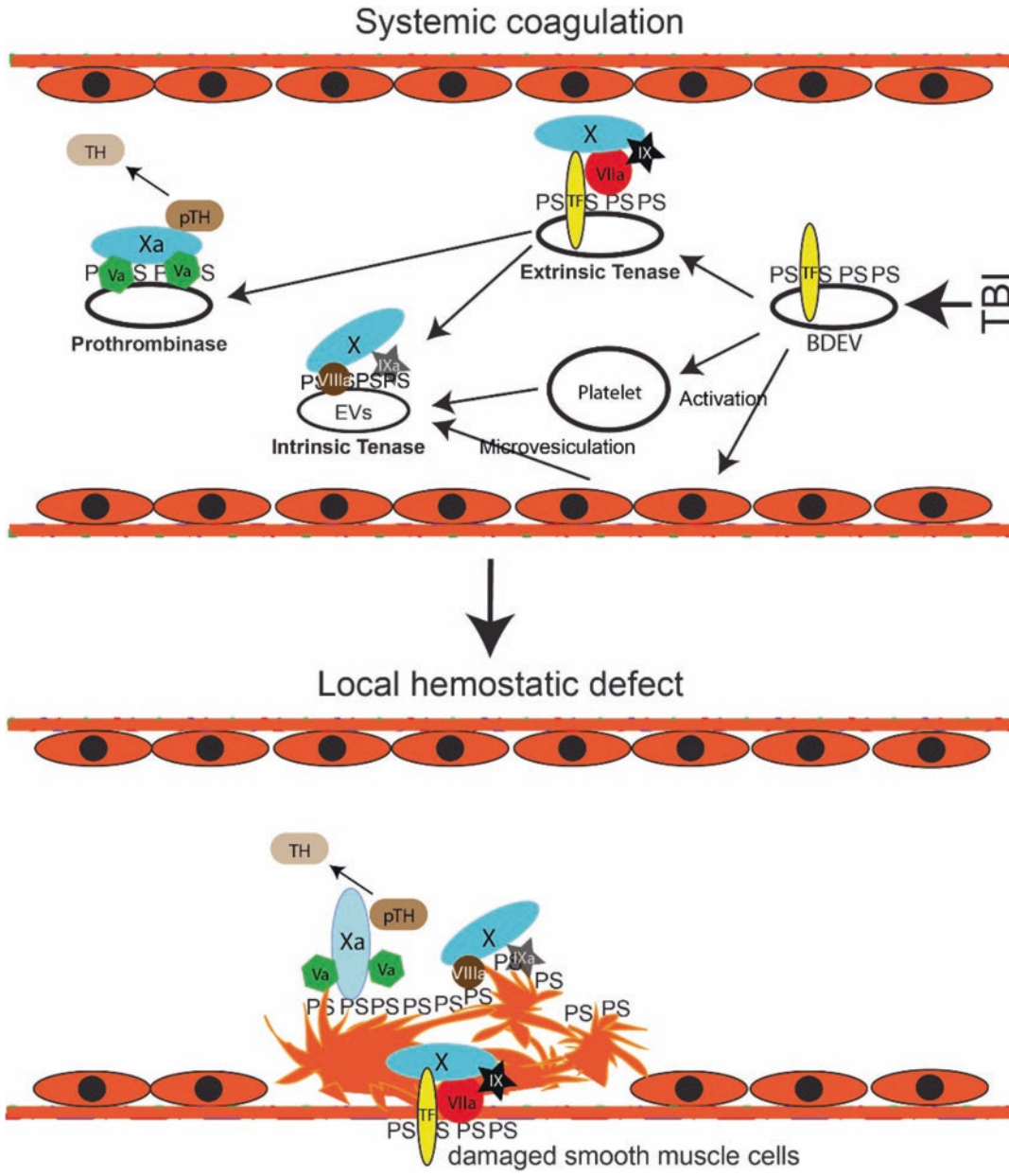


Fig. 34.3 Schematic illustration of how procoagulant extracellular vesicles could induce a systemic hypercoagulable state and localized hemostasis defect (bleeding). Top panel: Traumatically injured brain cells release TF- and PS-expressing BDEVs into circulation. These procoagulant BDEVs provide numerous circulating mini-platforms, on which the tenase complex is formed and activates factor Xa, which then forms the prothrombinase complex to catalytically activate prothrombin (pTH) to thrombin (TH). These BDEVs also activate platelets and endothelial cells to produce PS-expressing EVs that also promote thrombin generation. Bottom panel: Systemic coagulation on the surface of circulating EVs and activated platelets consume coagulation factors and platelets, resulting in insufficient hemostasis at the site of vascular injury. This EV-based coagulation could lead to non-focal, exaggerated systemic coagulation in the fluid phase and localized hemostatic defect at the site of injury, resulting in delayed intracranial or intracerebral hemorrhage

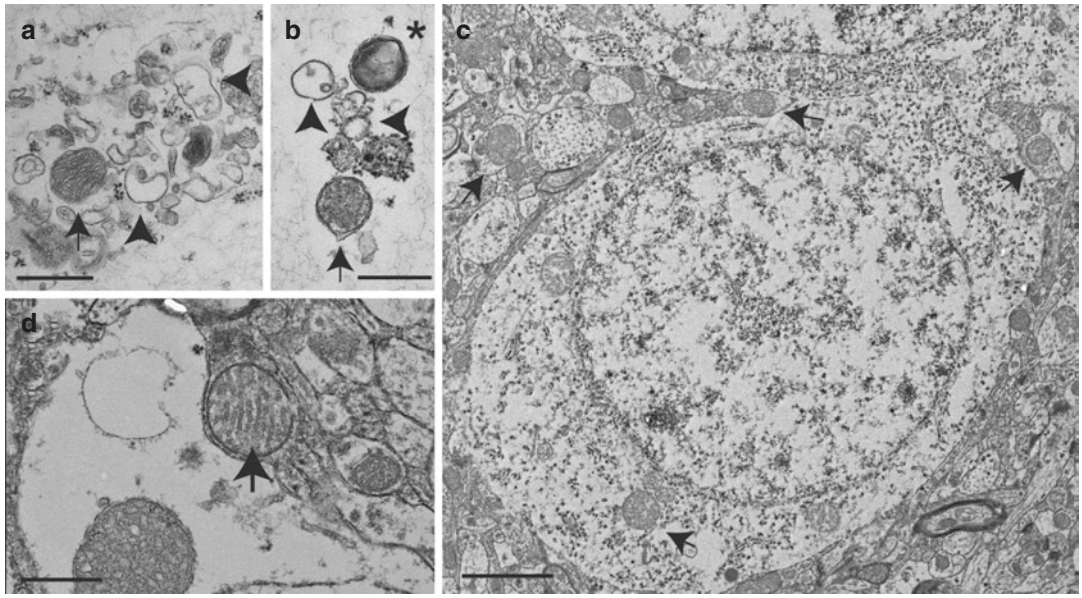


Fig. 34.4 Transmission electron microscopy images. (**a** and **b**) Extracellular vesicles collected from peripheral blood samples from mice subjected to severe traumatic brain injury. Structurally intact extracellular mitochondria (exMTs) are seen in both images (arrow: exMT; arrowhead: membrane vesicles; *an exMT that maintains the double membrane but has lost its content; bar = 500 nm). (**c**) A brain section from a non-injured mouse showing a large number of mitochondria with structures very similar to those found in the peripheral blood of TBI mice (arrow: mitochondria, bar = 500 nm). (**d**) An enlarged region of mouse brain showing structurally intact mitochondria (arrow, bar = 500 nm)

[44, 62] and endothelial cells [63] to express PS and release procoagulant EVs to further enhance and disseminate the TBI-induced hypercoagulable state. In addition to membrane EVs, more than 50% of annexin V^+ EVs found in the peripheral blood of mice subjected to severe TBI are intact or partially damaged extracellular mitochondria (exMTs), which are morphologically similar to intracellular mitochondria found in noninjured brains (Fig. 34.4) [64]. These exMTs promote coagulation through the mitochondrial anionic phospholipid cardiolipin [1,3-bis(sn-3′-phosphatidyl)-sn-glycerol] [64]. Cardiolipin is normally expressed in the inner membrane of mitochondria [65], but exposed on the surface of exMTs [64]. These circulating exMTs also promote inflammation by activating neutrophils to interact with endothelial cells [64, 66] and by serving as a substrate for phospholipase A2 group IIA [66], which is secreted during the acute phase reaction [67]. This phospholipase hydrolyzes the sn-2 acyl bond of glycerophospholipids to release potent proinflammatory free fatty acids and lysophospholipids. Finally, exMTs circulating in mice with acute TBI can be metabolically active to produce reactive oxygen species [62], which are known to activate platelets [62, 68, 69] and endothelial cells [70, 71]. The exMT-derived reactive oxygen species could become a significant source of oxidative stress because their activity persists longer in circulating blood that has only mildly reducing power [72–75], as compared to the oxidants released from the intracellular mitochondria, which can be rapidly neutralized in the highly reducing environment of the cytoplasm [76]. More importantly, these exMTs can bind target cells such as platelets [62] to localize the oxidative stress and evade blood-borne antioxidants. These exMTs therefore possess unique procoagulant, proinflammatory, and oxidative activities that differ from the intracellular mitochondrial dysfunction documented in disease states [77–80], including TBI [81–84], and from the proinflammatory

activity of molecules released from fragmented mitochondria [79, 80, 82, 85].

Despite their high procoagulant activity, BDEVs released into the circulation may be insufficient in numbers because TBI often involves a limited area of the brain in comparison to large injuries to the body and limbs, but they can serve as a trigger and amplifier of coagulation by activating other cells or proteins involved in all the four components of the hemostasis system.

Endothelial Injury

The endothelial barrier is considered a part of the hemostasis system because it provides an anticoagulant and platelet-repellent surface in the resting state [86, 87]. The anticoagulant endothelium becomes highly procoagulant and attracts platelets and leukocytes when it is activated or damaged by traumatic, ischemic, or inflammatory insults. A traumatic insult to the endothelium is often limited to the site of injury, but ischemic and inflammatory insults can spread to other parts of the brain and systemically, inducing TBI-IC and TBI-associated pulmonary and cardiac complications [88–90]. Multiple endothelial cell-derived molecules have been identified as markers for endothelial injury and some are associated with TBI-IC, including the adhesive ligand von Willebrand factor (VWF), the thrombin membrane cofactor thrombomodulin, intercellular adhesion molecule-1, vascular adhesion molecule-1, syndecan-1, E-selectin (CD62e), matrix metalloproteases, and endothelial cell-derived EVs [48, 91–97].

It is increasingly recognized that some of these molecules not only serve as biomarkers for endothelial injury but actively propagate endothelial injury and coagulopathy by making endothelium procoagulant (e.g., thrombomodulin [91, 98]) or releasing adhesive ligands (e.g., VWF) and proinflammatory mediators [99]. For example, thrombomodulin is a cofactor for thrombin-induced activation of protein C [100]. Thrombomodulin, protein C, and endothelial protein C receptor form the key anticoagulant system on the surface of endothelial cells. However, the extracellular domain of thrombomodulin sheds from activated endothelial cells [40, 101], including endothelial injury caused by TBI [102, 103]. The important questions are whether thrombomodulin shedding would weaken this anticoagulant system on the surface of the endothelium and whether soluble thrombomodulin remains active in the fluid phase of the blood. While not specifically examined in TBI, recombinant human thrombomodulin (rhMT, ART-123), which contains the extracellular domain of this transmembrane protein, is reported to reduce death by disseminated intravascular coagulation in patients with sepsis in some, but not in all studies [104–107]. Furthermore, both membrane-anchored and soluble forms of thrombomodulin are also found to inhibit fibrinolysis by enhancing the thrombin-induced activation of the thrombin-activable fibrinolysis inhibitor (TAFI) [108]. The question remains as to whether thrombomodulin acts as an anticoagulant or an antifibrinolytic agent during acute TBI. Syndecan-1 is a heparan sulfate proteoglycan that participates in the formation of glycocalyx on the surface of the endothelium [109]. Glycocalyx consists of multiple carbohydrate moieties of membrane glycolipids and glycoproteins, and it forms a protective layer on the apical surface of the endothelium (the vascular lumen) [110]. The shedding of syndecan-1 from the injured endothelium disrupts glycocalyx, contributing to the process of converting the anticoagulant endothelium into a highly procoagulant surface.

Extrinsic and Intrinsic Coagulation

The fibrinolytic product D-dimer and fibrinogen degradation products were detected in the peripheral blood samples of TBI patients within minutes of injury and followed first by a profound depletion of fibrinogen and then by prolonged prothrombin and partial thromboplastin times [7], indicating an early transition from a hypercoagulable to a hypocoagulable state [20, 111] due to the consumption of coagulation fac-

tors and platelets [112]. This consumptive TBI-IC is mechanistically distinct from the deficient and dilutional coagulopathy arising from extracranial trauma and hemorrhagic shock, as discussed in other chapters. The consumptive coagulopathy found in TBI patients can be reproduced in mice subjected to lateral fluid percussion injury [55, 64]. More importantly, noninjured mice develop a typical profile of consumptive coagulation after being infused with PS- and TF-expressing BDEVs (Fig. 34.5), demonstrating a key role of BDEVs in causing TBI-IC. Human TF is an integral membrane lipoprotein of 263 amino acids [113–115] and is enriched in the brain [116–118]. Its extracellular domain contains two fibronectin type III domains with two potential disulfide bonds of Cys49-Cys59 and Cys186-Cys209 [119] and forms a complex with the activated factor VIIa on the PS-rich membrane [120, 121]. The amino acid Cys245 in the cytoplasmic tail is linked to a palmitate fatty acyl chain [122], suggesting that TF is selectively concentrated in the lipid microdomains (lipid rafts) of the cell membrane [123–125]. This location allows TF to be enriched on the surface of BDEVs. As a result, extrinsic coagulation that normally occurs on the surface of activated platelets at the site of vascular injury can be initiated and propagated on circulating BDEVs and EVs from other cells (Fig. 34.3) [55], which can serve as mini-platforms to assemble the tenase complex [54, 126–129]. This EV-based coagulation would lead to nonfocal, exaggerated systemic coagulation in the fluid phase or on the surface of endothelial cells [116, 117] and a localized hemostatic defect at the site of the vascular injury. Consistently with the procoagulant activity of EVs and the development of TBI-IC, the apoptotic cell-scavenge factor lactadherin (milk fat globule–epidermal growth factor 8) [130] prevents a systemic hypercoagulable state and improves the neurological function and survival of mice subjected to severe TBI [131].

In comparison to studies on TBI-induced changes in coagulation, there are very few studies of how intrinsic anticoagulants such as tissue factor pathway inhibitor (TFPI), antithrombin, and protein C regulate the process of coagulation during acute TBI. TFPI is a single-chain polypeptide that blocks the early stages of coagulation by forming high-affinity and competitive complexes with the activated coagulation factors VIIa and Xa [132, 133]. As the primary inhibitor of the coagulation initiation, there is surprisingly little information on the role of TFPI in acute TBI-IC. In a prospective study of 120 patients with severe isolated TBI, the plasma levels of TFPI were found to be low in patients with coagulopathy, but this low TFPI level was not associated with the state of coagulopathy or clinical outcomes of the patients [134]. A similar observation was made in another smaller study [103]. The serine protease inhibitor antithrombin is a small glycoprotein produced by the liver and circulates as homologous α and β plasma forms, with α-antithrombin being the dominant form [135]. It inhibits most coagulation factors involved in the intrinsic coagulation pathway (the contact-activation pathway), including the activated FXa, FIXa, FXIa, and FXIIa. To a greater extent, antithrombin forms a complex with thrombin to block the proteolytic conversion of fibrinogen to fibrin. Heparin increases the inhibitory activity of antithrombin up to 1000-fold [136, 137]. The thrombin–antithrombin complex in the peripheral blood was found to be elevated during early injury in a small study of patients with severe isolated TBI [138], but its causal or regulatory role in the development of TBI-IC remains to be defined. Antithrombin given at 100 U/kg to patients with isolated TBI verified with CT scan did not appear to reduce injury-induced hypercoagulation [139]. Protein C is a plasma vitamin K-dependent glycoprotein zymogen [140, 141] that becomes activated serine protease upon binding to thrombin. Activated protein C proteolytically inactivates FVa and FVIIIa [142]. This anticoagulant activity is primarily detected on the surface of endothelial cells because activated protein C forms a complex with the endothelial protein C receptor and thrombomodulin [142]. Several studies have suggested that a maladaptive protein C pathway in response to brain injury and hemorrhagic shock causes a rapid APC-mediated coagulopathy [94, 143, 144]. APC has also been reported to protect the endo-

thelial integrity [145], independent of its anticoagulant activity [146].

Fibrinolysis

Thrombin cleaves fibrinogen to fibrin, which then quickly polymerizes to stabilize the platelet aggregate that is formed at the site of vascular injury to seal the wound [147]. The polymerized fibrin exposes noncompetitive high-affinity sites for tissue-type plasminogen activator (tPA) and plasminogen located in the αC-domain, which are cryptic in fibrinogen [148]. The D domain also undergoes conformational changes to expose low-affinity tPA- and plasminogen-binding sites [149]. tPA binds to these exposed sites to proteolytically activate the co-localized plasminogen to plasmin, which then cleaves crosslinked fibrin to dissolve the clot and reestablish the blood flow in an occluded vessel. This fibrinolytic process is kinetically slow during hemostasis because tPA is released slowly and has limited access to fibrin polymers trapped in a platelet clot. This delay in plasminogen activation is necessary to ensure complete hemostasis at the site of vascular injury before fibrinolysis is initiated. However, the sequential changes in laboratory measurements of patients with TBI-IC indicate very early development of the hyperfibrinolytic state [7], raising the question whether TBI-induced hyperfibrinolysis is initiated independently of fibrin formation. This alternative fibrinolysis remains to be experimentally and clinically verified. One interesting hypothesis is that fibrinolysis is triggered early and enhanced significantly by fibrin polymers formed on the surface of much smaller circulating EVs. This notion is consistent with several clinical and laboratory observations. First, plasma tPA and the fibrinolytic product D-dimer are associated with progressive hemorrhage [150] and poor clinical outcomes for patients with TBI [151]. Second, tPA deficiency reduces persistent intracerebral hemorrhage but does not prevent systemic coagulopathy in TBI mice [152].

There are fewer reports on the role of fibrinolysis inhibitors in the development of TBI-IC. The plasmin inhibitor α2 macroglobulin decreases significantly within 2 hours and then increases by 4 hours in plasma samples collected from rats subjected to polytrauma [153]. Mice deficient in plasminogen activator inhibitor-1 (PAI-1) developed severe intracerebral hemorrhage after being subjected to severe TBI [152]. Interestingly, a PAI-1 increase was detected along with elevated plasmin activity and high levels of plasma plasminogen and D-dimers in mice subjected to polytrauma [153], suggesting that TBI can induce an imbalance between fibrinolysis and its inhibition. This imbalance is evident in studies showing that trauma patients with hyperfibrinolysis had significantly increased levels of tPA, but unchanged [154] or even reduced levels of PAI-1 [155], as compared to those without hyperfibrinolysis. Tranexamic acid, a synthetic derivative of the amino acid lysine that reversibly blocks the lysine-binding sites on plasminogen, reduced bleeding in trauma patients in a randomized, double-blind, placebo-controlled trial (CRASH-2) [156]. The recently completed CRASH-3 trial (NCT01402882) further shows that tranexamic acid (1 gm loading dose IV followed by 1 gm over 8 hours) also reduced the risk of death in patients with mild-to-moderate TBI (GCS 9–15, RR 0.78 [95% CI 0.64–0.95]), but not in those with severe TBI (GCS 3–8) [157]. This randomized, placebo-controlled trial further shows that tranexamic acid was more effective when it was given within 3 hours of injury to patients with mild-to-moderate TBI and did not increase the risk of thrombosis and seizure. However, this trial did not report whether tranexamic acid specifically reduced the development and severity of TBI-IC.

Platelets and Adhesive Ligands

Among the four components of hemostasis (Fig. 34.1), platelets are least understood for their role in the development of TBI-IC. Platelets have moderately low counts, enhanced activation and aggregation, and high procoagulant activity in TBI patients and in rats subjected to TBI [158–162]. These observations suggest that platelets

are either activated or primed for activation during acute TBI [26, 163, 164]. Activated platelets express procoagulant anionic phospholipids and also release EVs [62, 98]. These platelet-derived EVs are enriched in anionic phospholipids and are 50- to 100-fold more active in promoting coagulation than activated platelets themselves are [54, 118, 165]. Consistent with this platelet hyper-activity, cerebral microthrombi are detected in the peri-contusion cortex [158, 166] and contain not only fibrin [167] but platelets [166, 168] and von Willebrand factor (VWF) [166]. Activated platelets can either adhere to endothelial cells and leukocytes or are cleared from the circulation, resulting in thrombocytopenia that has been widely reported in patients with TBI and predicts intracranial hemorrhage progression and death [169, 170]. However, how platelets are activated or become hyper-reactive has not been studied mechanistically. A recent study suggested that metabolically competent exMTs released during acute TBI could bind platelets through the phospholipid receptor CD36 and activate platelets by reactive oxygen species released from these exMTs [62]. Another factor potentially responsible for the platelet phenotype found in acute TBI is the platelet-activating factor (PAF: 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine), which has been studied extensively for its neurotoxicity but not for causing TBI-IC. PAF is enriched in the brain and spinal cord [171] and is released during cerebral ischemia [172, 173]. Platelets have both high-affinity and low-affinity receptors for PAF, but it is the PAF binding to the high-affinity receptor [174] that activates platelets by initiating intracellular signals to activate phospholipases C and A2, which hydrolyze phosphoinositide to release the potent platelet activator arachidonic acid [170]. PAF released from injured brains is likely to be EV-bound because it is linked to membrane lipid microdomains. PAF levels have indeed been reported to increase up to 20-fold in experimental models of ischemia and reperfusion injury to the spinal cord [175]. Experimental PAF antagonism attenuates ischemic edema, early postischemic hyperemia, and microvascular thrombosis in animal models [26].

Another observation that has not been fully explained is that platelets from TBI patients [159–161, 176] and from experimental TBI rats [160, 177, 178] and swine [179] respond poorly to adenosine diphosphate and to arachidonic acid. This poor response is independent of platelet counts, hemorrhagic shock, and tissue hypoperfusion [159, 160, 177, 178]. More importantly, it does not appear to be caused by granule depletion of activated platelets [180], strongly suggesting a new TBI-induced platelet phenotype that has not been mechanistically examined.

Despite extensive evidence of platelet activation during acute TBI, studies of adhesive ligands that activate platelets are very limited. Among these ligands is VWF, which has been widely used as a marker for endothelial injury, but its causal role in TBI-IC has just begun to emerge [98]. VWF is synthesized exclusively in megakaryocytes and endothelial cells as a single-chain propolypeptide of 2813 amino acids [181] that contains four types of repeated domains in the order D1-D2-D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK (Fig. 34.5a) [182–187]. This domain structure has recently been updated with more defined structural motifs [188]. Newly synthesized pro-VWF first dimerizes through the CK domain [189]. A variable number of dimers then multimerize through N-terminal disulfide bonds after proteolytical removal of the large propeptide [190, 191]. This multidomain multimeric structure enables VWF to interact simultaneously with multiple receptors on the same cell or different cells. After synthesis, VWF multimers are either constitutively released into the circulation or stored in the Weibel-Palade bodies of endothelial cells and the α-granules of megakaryocytes/platelets, where multimerization is likely to continue [192, 193]. As a result, VWF multimers in these storage granules are enriched in ultra-large forms called ULVWF multimers (Fig. 34.5b), which are released upon activation of endothelial cells and platelets [194–196]. These ULVWF multimers differ from VWF multimers found in the plasma at the resting state not only in molecular mass but also in adhesive activity [181]. Plasma VWF multimers bind platelets poorly unless they are immobilized on the subendothelial collagen at the site

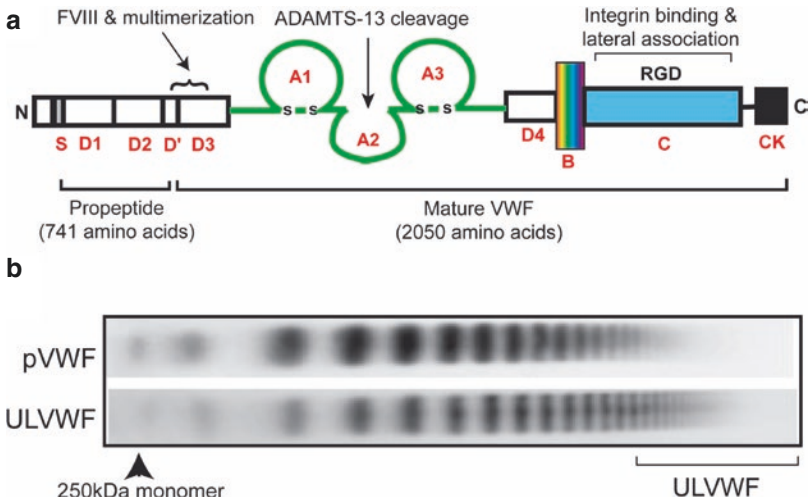


Fig. 34.5 (**a**) Schematic illustration of the VWF domain structure and function. The D'D3 domains bind and protect coagulation factor VIII from proteolysis and also mediate VWF multimerization; the A1 and A3 domains are disulfide bond-linked loops that bind the platelet receptor GP Ib-IX-V complex and the subendothelial collagen, respectively; the A2 domain contains the peptide bond Y^{1605}-M^{1606} cleaved by ADAMTS-13; the C domain contains an integrin-binding RGD sequence and free thiols that are involved in the lateral association of VWF multimers to form hyper-adhesive strings; and the CK domain contains the sites for VWF dimerization. (**b**) VWF multimers found in normal plasma of healthy subjects and those freshly released from activated endothelial cells before being cleaved by ADAMTS-13 (ULVWF)

of vascular injury or activated by high fluid shear stress [194–196], suggesting that they circulate in an inactive conformation. By contrast, ULVWF multimers freshly released from the storage granules are intrinsically hyper-adhesive and spontaneously bind platelets and endothelial cells [191, 197–199]. The structural difference between the two forms of VWF is that the platelet-binding A1 domain is hidden in the globular structure of plasma VWF but exposed on the surface of hyper-adhesive ULVWF [200, 201]. Upon release, these hyper-adhesive ULVWF multimers are rapidly cleaved at the Y^{1605}-M^{1606} peptide bond in the A2 domain by the metalloprotease ADAMTS-13 (*A Disintegrin And Metalloproteinase with a Thrombo Spondin type 1 motif, member 13*) to become VWF multimers that are found in circulation in the resting state [202–204]. The proteolysis serves as a safeguard to prevent spontaneous VWF-platelet interaction, which induces thrombosis and thromboembolism, while maintaining the hemostatic activity of plasma VWF at the site of vascular injury. This safeguard can be disrupted during acute injury when the rate of ULVWF release from injured endothelial cells overwhelms the capacity of ADAMTS-13 to cleave it, or when VWF multimers are oxidized to resist cleavage [205] or form laterally associated fibrils that are highly adhesive in binding platelets and endothelial cells [182, 206]. Consistent with the notion, exogenous ADAMTS-13 reduces TBI-IC without impairing the baseline hemostasis and improves the neurological function and survival of the mice subjected to severe TBI [98].

The impact of VWF hyper-adhesiveness on the pathophysiology of TBI is further demonstrated by the following observations. First, high plasma levels of VWF are associated with poor outcomes of patients with TBI [91, 207, 208]. Second, low ADAMTS-13 activity is associated with coagulopathy, endothelial damage, and mortality in patients with severe trauma [209], including TBI [210]. Third, VWF-containing thrombi are detected in the lesion boundary zone and remote regions of the cortex within hours of TBI in a rat model [166]. Fourth, VWF deficiency upregulates the tight junction protein claudin 5 to protect the integrity of the blood–brain barrier

[211]. Fifth, a VWF-A1 antibody blocks inflammation-induced vascular leakage [212]. Finally, microglial activation and neuronal injury after subarachnoid hemorrhage are reduced in VWF-deficient mice and in mice infused with ADAMTS-13 [213].

Clinical and Histological Presentation

TBI-IC is commonly manifested as secondary or delayed intracranial and intracerebral hemorrhage and associated injuries on repeated CT or MRI scans (Fig. 34.6), as well as an intraoperative finding of difficulty in hemostasis during the decompression procedures [214, 215]. These secondary injuries can either progress from the original lesion or develop in new locations. In a review of 253 patients with serial CT scans, the risk of developing delayed cerebral injury was found to be 85% for TBI patients who had one or more abnormal hemostatic tests upon admission to the emergency room, as compared to 31% for those without initial abnormal laboratory findings [216]. Progressive intracranial hemorrhage is significantly more common in TBI patients with laboratory-defined coagulopathy upon admission to the emergency room and carries a fivefold higher risk of death [217]. In another study of 142 TBI patients, repeated neuroimaging scans taken within the first 2 hours post injury detected 48.6% of patients with progressive intracranial hemorrhage, which was best predicted by a prolonged activated partial thromboplastin time [218].

In addition to intracranial and intracerebral hemorrhages, cerebral microvascular thrombosis has also been reported [158]. In a study of 265 patients with moderate or severe TBI, cerebral tissue infarction was detected by CT scan in 10% of patients in the first week post injury and was associated with thrombocytopenia (OR 2.2; CI 95% 1.1–4.7), a prolonged prothrombin time (OR 3.241, 95% CI 1.090–7.648), and elevated levels of the plasma D-dimer (OR 7.260, 95% CI 1.822–28.076) [219]. These clinical observations are supported by the findings that microvascular thrombi are detected in the brains of rats subjected to either lateral fluid percussion injury or controlled cortical impact injury, and pigs subjected to head rotational acceleration injuries [158, 166]. This apparent discrepancy between progressive hemorrhage and microvascular thrombosis in the brain is consistent with consumptive coagulopathy derived from an injury-induced hypercoagulable state.

Severe TBI induces not only systemic coagulopathy but dysfunctions in other organs. In a study of 154 patients with severe TBI (GCS $\leq$ 8), nonneurological complications were identified as respiratory pathologies (61%), dyselectrolytemia (46.1%), cardiovascular abnormalities (34.4%), sepsis (26%), abdominal complications (17.5%),

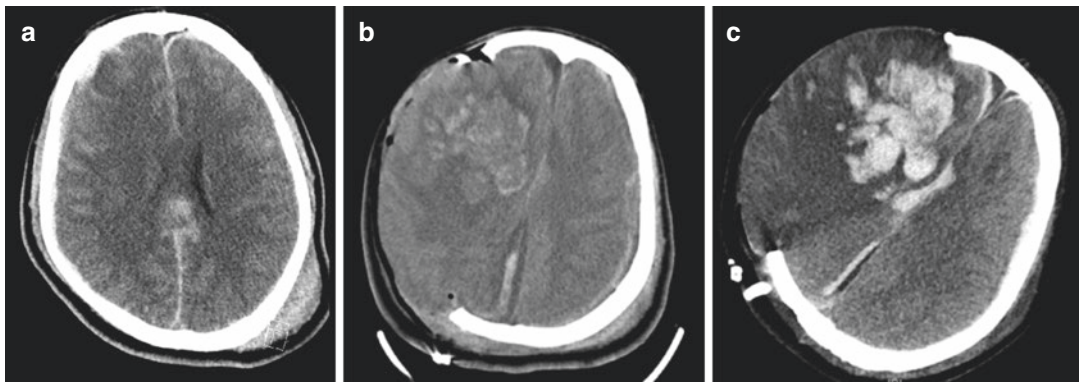


Fig. 34.6 Secondary intracerebral hematoma. A 30-year-old male suffered from a motor vehicle collision. A CT scan 6 hours after injury shows cerebral contusion and midline shift (**a**). The patient underwent decompressive craniectomy (**b**) and developed severe cerebral swelling and progressive intracerebral hemorrhage (**c**)

and acute kidney injury (3.9%) [88]. Most of these complications were associated with poor clinical outcomes for the patients. Respiratory complications are most common and can present as acute lung injury, acute respiratory distress syndrome, pneumonia, pleural effusions, pulmonary edema, and pulmonary thromboemboli [89]. Severe TBI (AIS head > 2) has been associated with significantly greater odds of developing acute respiratory distress syndrome, acute lung injury, and ventilator-associated pneumonia in the post hoc analysis of data obtained from the Pragmatic, Randomized Optimal Platelets and Plasma Ratios (PROPPR) trial [90]. These clinical observations are again supported in rodent models of severe TBI, in which pulmonary microvascular fibrin deposition, microthrombosis, and interstitial bleeding are detected [55, 168]. Despite the evidence of multiple organ injuries secondary to TBI, it is not known whether these nonneurological complications develop independent of TBI-IC, contribute to the development of TBI-IC or are caused by TBI-IC.

A demographic shift in TBI patients from being predominantly young to increasingly older (≥50 years) has resulted in more patients with comorbidities or receiving antithrombotic, anticoagulant, and antiplatelet medications prior to TBI [220, 221]. These preconditions make differential diagnosis of TBI-IC more difficult, but also necessary because patients with comorbidities or taking these medications require more frequent monitoring for the development of delayed intracrainal and intracerebral hemorrhage [222, 223]. They also carry a significantly greater risk for systemic complications and have poor clinical outcomes [224–226].

Laboratory Measurements

TBI-IC is primarily defined by laboratory tests, which measure different aspects and stages of hemostasis. Table 34.1 lists the laboratory tests that are currently used to assess hemostasis in patients with TBI, as reported in the literature. These tests detect the components involved in the different stages of primary hemostasis. For example, in a study of 972 TBI patients who developed coagulopathy, D-dimer and fibrin degradation products were detectable within minutes of injury followed first by profound depletion of fibrinogen and then by prolonged prothrombin and partial thromboplastin times [26]. This sequence of events indicates a transition from a hypercoagulable to a hypocoagulable state [227] and also explains why the prevalence of TBI-IC defined by these tests varies significantly among studies. Most importantly, it distinguishes TBI-IC mechanistically from deficient and dilutional coagulopathy induced by injury to the body and limbs and hemorrhagic shock [228]. To address the limitations of individual tests, viscoelastic devices such as thrombelastometry (ROTEM) and thromboelastography (TEG) are increasingly used to globally evaluate hemostasis in TBI patients. These devices measure the viscoelastic properties of blood clot to define clot formation, stability, and strength [229, 230]. They detect various changes in blood samples from TBI patients, but functional linkages of these changes to traditional hemostatic tests remain poorly defined. Furthermore, these devices do not adequately measure platelet function and lack any direct means of evaluating endothelial injury. The DIC score originally developed by the Interactional Society of Thrombosis and Hemostasis [231] has also been used to define TBI-IC in some studies [7, 10, 232, 233]. This score system integrates measurements of platelets, fibrinogen/fibrin, and prothrombin time, but not those of endothelial injury. Its value for defining TBI-IC also needs to be more precisely evaluated. Although most studies focus on coagulopathy induced by severe TBI, a recent study of a small cohort of 73 patients who developed mild and isolated TBI (GCS 14–15) and were not on antiplatelet and anticoagulant medication also found that elevated plasma levels of the fibrinolytic product D-dimer independently predicted the cerebral injury detectable by CT scan [234].

One interesting note is that the current laboratory tests for TBI-IC are overwhelmingly focused on coagulation and fibrinolysis. Platelets and their adhesive ligands such as VWF have not been mechanistically studied for their role in the

development of TBI-IC. This is surprising, given that platelets and adhesive ligands are the key components of hemostasis and are known to be altered by TBI. Similarly, the disruption of endothelial integrity by traumatic and inflammatory injuries has been extensively studied for cerebral edema and ischemic injury secondary to TBI, but the role of endothelial dysfunction in the pathogenesis of TBI-IC has not been mechanistically defined even though these injuries can make the endothelium procoagulant. In one study, patients with TBI-IC were found to have lower plasma levels of soluble thrombomodulin and higher levels of soluble syndecan-1 than patients without coagulopathy [235]. Plasma syndecan-1 shed from the injured or activated endothelium may be associated with TBI-IC because it indicates the disruption of the protective glycocalyx found in TBI patients and in experimental rats subjected to severe TBI [236, 237].

Despite the widespread use of these laboratory tests to define TBI-IC, key knowledge gaps remain regarding their predictive and diagnostic values. First, current laboratory tests diagnose coagulopathy after it occurs but none can predict when or if it will occur. Second, these tests have proven valuable for predicting the impact of coagulopathy on the outcomes of TBI, but there is no consensus on whether prophylaxis measures should be considered on the basis of their results. Third, these tests have found more TBI patients in a coagulopathic state, but only a fraction of those patients actually developed secondary or delayed intracranial or intracerebral hemorrhage [14, 15, 238], suggesting that other unidentifed modifiers or regulators exist. Comprehensive evaluation and standardization of these laboratory tests is therefore needed to identify tests that can predict the occurrence and severity of clinical TBI-IC. Using an assay-guided algorithm combined with artificial intelligence may help in the creation of individualized models for predicting TBI-IC. In a pilot study, Gratz et al. used a thromboelastometric-guided algorithm to identify 21 of 32 patients as having TBI-IC, as compared to only 5 using conventional coagulation tests [238], but the predictive value of this algorithm remains to be investigated.

Clinical Management

Decompression surgery remains the treatment of choice for patients with a significant size of secondary or delayed intracranial and intracerebral hematoma. However, it is debatable as whether laboratory-defined TBI-IC requires specific treatments perioperatively. For example, an AAST-MITC propensity score analysis found that the recombinant factor VIIa did not improve the clinical outcomes of 129 TBI patients (GCS $\leq$ 13) [239]. The antifibrinolytic tranexamic acid was found to be effective in reducing hemorrhage in trauma patients in a randomized, double-blind, placebo-controlled trial [156]. Findings from the recently completed CRASH-3 trial suggest that tranexamic acid also reduces death in patients with mild-to-moderate TBI (GCS 9–15), especially when it is given within 3 hours of injury [157]. This large trial helps resolve some of the uncertainties related to the efficacy of tranexamic acid for TBI [240–242]. The lack of definitive guideline for managing TBI-IC could hinder the progress toward more efficient prevention and targeted therapies for this lethal TBI complication. For example, the timely, judicious, and balanced use of blood component and fluid resuscitations has been the management choice to correct the deficient and dilutional coagulopathy associated with extracranial trauma and hemorrhagic shock. Despite the fact that patients with isolated TBI do not lose significant amounts of blood, and therefore have no intrinsic need for transfusion, they are routinely transfused with blood products to treat coagulopathy, to reverse the effects of anticoagulant and antiplatelet medications, and to correct comorbidities. The efficacy of blood transfusions has been evaluated in only a few clinical outcome studies, which were mostly retrospective and had very limited sample sizes, patient stratification, and confounding adjustments. Nevertheless, these studies have found that transfusion of blood products to patients with isolated TBI is often ineffective and potentially detrimental [243], in part because it could propagate the TBI-induced hypercoagulable state by supplying more coagulation factors and proco-

agulant extracellular vesicles accumulated in the stored blood products. Basic and translational studies at the cellular level are needed to understand the impact of blood component transfusions on endothelial injury, coagulopathy, hyperfibrinolysis, and platelet dysfunction in patients with TBI-IC.

Patients on antithrombotic and antiplatelet medications prior to TBI require effective strategies to revise their bleeding diathesis before and during surgery [220, 221]. The early use of prothrombin complex concentrate (PCC) is recommended for patients on warfarin [244] with a target INR of 1.5. PCC is also used for patients on factor X inhibitors [245], with the treatment goal of a modified aPTT of <90. Direct thrombin inhibitors such as dabigatran are not effectively reversed by PCC, but could be reversed by the targeted antidote idarucizumab.

Clinical Outcome

Coagulopathy upon arrival at the emergency room has been consistently associated with poor clinical outcomes or death in TBI patients [9, 14–16, 19, 219, 246], primarily because coagulopathy significantly increases the risk of continuous hemorrhage or the development of delayed intracranial or intracerebral bleeding [11, 12]. In one report, TBI patients who developed coagulopathy within 24 hours of injury had a mortality rate of 55%, as compared to 23% in those who developed hemostatic abnormalities after 24 hours [20]. Uncontrolled coagulopathy in severe TBI increases the risk of intraoperative severe brain swelling 11.5-fold [247]. In a systematic review of 22 published reports, the mortality of patients with isolated TBI and coagulopathy defined by various laboratory tests was found to range from 17% to 86% [14]. In the meta-analysis of 34 studies discussed early, the risk of death in patients with coagulopathy was found to be approximately ten times as high as in those without coagulopathy, and surviving patients are more than 30 times as likely to have poor clinical outcomes if coagulopathy is present upon emergency room arrival [9]. In the same TR-DGU study [15], overall hospital mortality was 50.4% among 706 patients with isolated TBI who developed coagulopathy upon arrival, as defined by the prothrombin time test (Quick's value) <70% and/or platelet counts of <100,000/μl. Even with comparable injury severity at admission, TBI patients with coagulopathy as defined by INR had significantly higher in-hospital mortality than those without [248]. A recent analysis of 591 patients with isolated TBI, platelet counts of $\leq 100 \times 10^9$/l, and INR of >1.5 was associated with progression of the initial insults on repeated CT scans and predicted the need for surgery and the likelihood of death [249].

Conclusion

Coagulopathy is a frequent and life-threatening complication of TBI. It is consumptive in nature and involves all four components of the hemostasis system. The underlying mechanism of TBI-IC remains poorly understood. Clinical diagnosis varies depending on individual laboratory tests and therefore requires standardization. Because of these critical knowledge gaps, clinical management options for TBI-IC remain limited and often ineffective. Basic science research and large clinical trials are needed to more precisely define TBI-IC, develop more accurate predictive markers, and identify effective and targeted treatments.

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35

Blunt Solid Organ Injury: A Call for Timely Chemoprophylaxis in the Setting of Early Hypercoagulability

Julia R. Coleman and Fredric M. Pieracci

Introduction

Trauma is one of the leading causes of death in persons aged 1–44 years, and among those aged 15–25 years, the majority of mortality is due to blunt trauma from motor vehicle collision [1]. An average of 90 people die each day in motor vehicle crashes in the United States, often due to blunt solid organ injury (BSOI) [2]. Beyond the high mortality of patients with BSOI, there is significant morbidity in the form of millions of annual emergency department (ED) visits for treatment of nonfatal BSOI injuries [3]. The most frequently injured organ is the liver, followed by spleen and kidney, followed by the pancreas and adrenal glands [4, 5]. These solid organ injuries can be commonly associated with bleeding requiring surgical management, with a range of 20–30% of BSOI patients undergoing operative intervention [6–9]. Typical trauma service management protocols include urgent operative interventions for patients with BSOI and hemodynamic instability and/or ongoing transfusion requirements, endovascular intervention for BSOI with associated arterial injury and active extravasation, and nonoperative management (NOM) for venous injuries [6, 10].

Over the last few decades, in the setting of widespread adoption of endovascular procedures, there has been a paradigmatic shift toward NOM of BSOI patients [6, 11]. Despite endovascular advancements, the incidence of failure of NOM remains an issue for BSOI patients, with rates ranging from 2% to 25% [12–14]. Due to the occasional failure of NOM from bleeding, there is continued debate about the optimal time to initiate venous thromboembolism (VTE) chemoprophylaxis in injured patients with nonoperatively managed BSOI.

The coagulation profile of BSOI is poorly understood, but pathologic hypercoagulability has been described, with high rates of VTE ranging from 9% to 13%, even in some cases despite VTE chemoprophylaxis [15–17]. Several studies of BSOI patients detail a robust hypercoagulable profile which may be driving this high rate of thrombotic morbidity, characterized by shorter time to clot formation, greater clot propagation, and greater clot strength, which is present as early as hospital admission [16, 18, 19]. The concept of bleeding BSOI patients being hypercoagulable is somewhat counter-intuitive, and a misunderstanding of this apparent paradox is in large part responsible for widespread hesitancy among surgeons to initiate VTE chemoprophylaxis in these patients. However, while the

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mechanism of this hypercoagulable state has not been elucidated, the clinical consequences, including thrombotic morbidity such as venous thromboembolism (VTE; including deep venous thrombosis [DVT] and pulmonary emboli [PE]), can occur in up to 50% of trauma patients without chemoprophylaxis and 4–30% of trauma patients with chemoprophylaxis [20–26]. Ultimately, the benefit of mitigating thrombotic risk must be balanced against the risk of exacerbation of intraabdominal hemorrhage leading to failed NOM. This chapter examines existing knowledge about the unique trauma-induced coagulopathy (TIC) phenotype of BSOI patients and mitigation strategies for this hypercoagulability-associated thrombotic risk. Management of traumatic brain injury patients is specifically covered in a separate chapter.

Coagulation Profile of BSOI

Characterizing the coagulation profile of BSOI along the spectrum of TIC phenotypes is essential, in particular given the high prevalence of BSOI among trauma patients [2]. TIC is known to be a dynamic process, with the majority of severely injured patients transitioning from an initial hypocoagulable state to a hypercoagulable state [27, 28]. Delineation of the timing of the transition to a hypercoagulable state among BSOI is essential to inform optimal VTE chemoprophylaxis strategies and attenuate the risk of thrombosis without concomitantly increasing the risk of bleeding leading to failure of NOM. Despite the first description of the dynamic transition from hypocoagulability to hypercoagulability in severely injured patients in the early 1900s, the precise timing of this change remains uncertain and is likely influenced by a myriad of variables, including degree of shock and tissue injury and resuscitation such as blood product transfusion [29–31]. A retrospective review of all nonoperative BSOI patients at a single level-1 trauma center from 2009 to 2012 examined the thrombelastographic profiles of BSOI patients and found that no BSOI patients presented with a hypocoagulable profile (defined by prolonged activated clotting time, decreased rate of propagation, and decreased clot strength) [19]. Further, Chapman et al. found that the majority of BSOI patients rapidly converted to a hypercoagulable profile, characterized by elevated clot strength (G, calculated from maximum amplitude [MA]), by 48 hours (Fig. 35.1) [19]. In a similar retrospective study of serial rotational thrombelastometry (ROTEM) over 120 hours in critically injured patients, Sumislawski et al. found that nearly half of patients converted to a hypercoagulable profile, as assessed by maximum clot firmness on ROTEM (analogous of MA on TEG) by 120 hours [18].

A recent biinstitutional study of all BSOI patients at two level-1 trauma centers built on these previous bodies of work through a prospective analysis [16]. Serial citrated kaolin TEGs

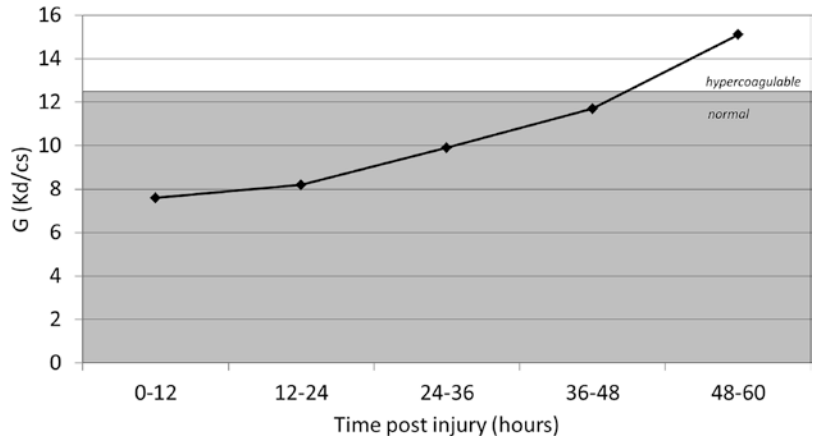


Fig. 35.1 G values, an effect of maximum amplitude. The G value (normal range, 5.2–12.4 Kd/cs) crosses into the hypercoagulable range at 48 hours

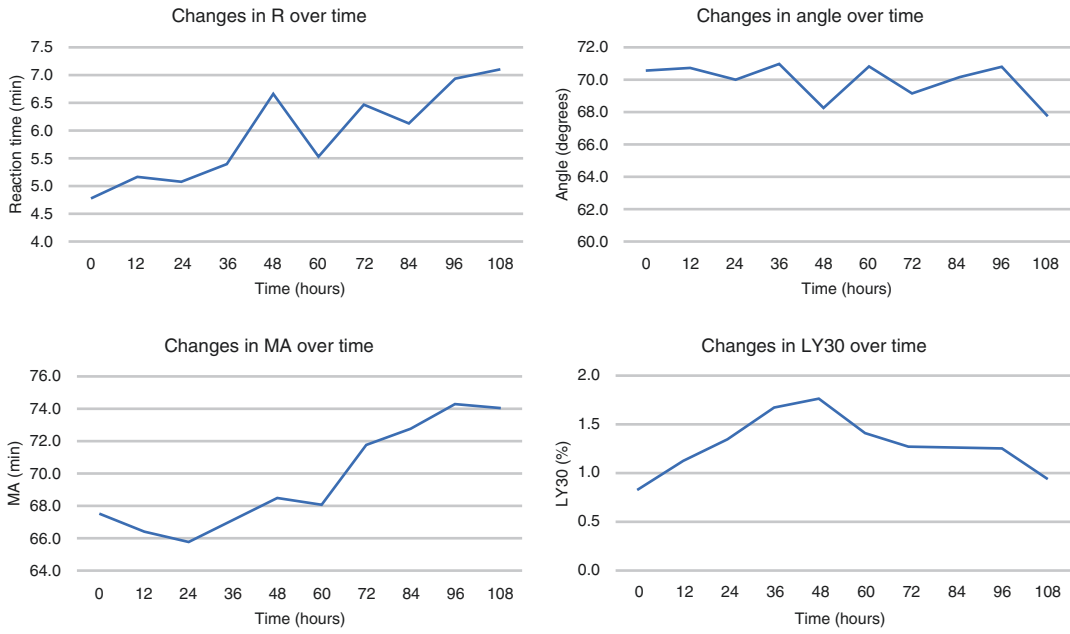


Fig. 35.2 Changes in thrombelastographic profiles over time in BSOI patients. MA maximum amplitude, LY30 fibrinolysis 30 minutes after MA

were performed on all nonoperatively managed BSOI patients every 12 hours for 108 hours. Remarkably, all BSOI patients were hypercoagulable upon admission to the ICU: 88% by reaction time, 66% by angle, and 33% by maximum amplitude (measure of clot strength). Additionally, 50% of patients were in fibrinolysis shutdown (with no BSOI patients in hyperfibrinolysis upon ICU admission). This initial hypercoagulability persisted in the majority of patients for the remainder of blood sampling (100% at 108 hours; Fig. 35.2).

This study also included an analysis of tPA-challenge TEG to address the degree of tPA-mediated fibrinolysis via the tPA-challenged TEG, which adds exogenous tPA at a concentration of 75 ng/mL to whole blood before performing citrated native TEG. tPA resistance has been linked to a five-fold increase in mortality in severely injured patients [32]. Upon ICU admission, 50% of patients were tPA-resistant, and 43% remained so at 108 hours [16]. Further, patients who had a thrombotic complication had a longer median time to chemoprophylaxis, as well as lower degree of fibrinolysis at 12 hours, greater clot strength (MA) at 48 hours, and a greater degree of tPA resistance at 84 hours compared to those without clot complications. Not only do these data highlight the importance of identifying objective markers of pathologic hypercoagulability in BSOI patients, but also underscore the importance of prompt VTE chemoprophylaxis, as delay in initiation has been linked to higher VTE rates in other investigations of trauma patients [33]. Interestingly, the hypercoagulability in BSOI patients is pervasive despite many of these patients presenting in shock, which contradicts historical dogma that shock is intrinsically linked to hypocoagulability and bleeding risk. In summary, the majority of BSOI patients either present to the ICU already hypercoagulable or become so soon after admission.

Mechanism Behind BSOI-Associated Hypercoagulability

While BSOI-associated hypercoagulability has been described in several retrospective and prospective studies, the mechanism behind this TIC phenotype has not been elucidated. Animal mod-

els of isolated tissue injury result in a hypercoagulable profile (shorter time to clot formation, greater rate of clot propagation and strength) with fibrinolytic shutdown, whereas isolated hemorrhagic shock manifests a hypocoagulable, hyperfibrinolytic profile [34, 35]. This hypercoagulability has been attributed to the release of various proteins in the setting of tissue injury, which affects the hemostatic and fibrinolytic process. While concomitant shock can lead to profound coagulopathy, specifically through upregulation of thrombomodulin expression on the endothelium in the setting of tissue hypoperfusion [36], tissue injury alone can lead to hypercoagulability, in the form of increased thrombin generation and endotheliopathy with inflammatory cascade signaling [36–38]. Tissue factor released in the setting of tissue and endothelial injury propagates thrombus formation and fibrin generation, ultimately potentiating platelet aggregation downstream [39]. A prospective study of 404 trauma patients admitted to a level 1 trauma center including thrombelastography data and plasma markers of shock and endotheliopathy found that soluble E-selectin, a marker of endothelial activation, was independently associated with a more hypercoagulable TEG in the form of shortened activating clotting time, increased angle, and increased MA [38]. Clinical studies have also indicated increased release of myosin into circulation after tissue injury [40], and skeletal muscle myosin in vitro is known to have a procoagulant profile, specifically through augmentation of fibrin and platelet deposition [41]. In addition to release of myosin, damage-associated molecular patterns (DAMPs) are also released in the setting of tissue injury; of those, histones are known to increase phosphatidylserine surface expression on red blood cells, accelerate thrombin formation, and propagate endothelial damage (recruiting more platelets and fibrin) when released to the extracellular space [42–45]. Lastly, tissue trauma also leads to activation of the sympathoadrenal system, resulting in higher levels of catecholamines, which can cause endothelial damage, glycocalyx degradation, and exposure of tissue factor and collagen, ultimately resulting in a hypercoagulable state [46, 47]. While the precise mechanisms underlying the hypercoagulability of BSOI are unknown, elucidating the unique drivers of BSOI-associated hypercoagulability may allow for targeted, individualized chemoprophylaxis strategies and/or thrombotic risk mitigation. Ultimately, the tissue injury of solid organ injury and the resultant hypercoagulable TIC phenotype call into question the current practice of delaying VTE chemoprophylaxis in BSOI patients due to concerns for exacerbation of injury-related bleeding and failure of NOM.

Risk Mitigation: VTE Chemoprophylaxis Strategies in BSOI-Associated Hypercoagulability

The optimal timing of VTE chemoprophylaxis in BSOI patients remains unsettled. Results of a Southwestern Surgical Congress Multicenter trial indicate that there is considerable variation in VTE chemoprophylaxis strategies across trauma centers [48]. Furthermore, the Eastern Association for the Surgery of Trauma guidelines for NOM of both hepatic and splenic injuries concluded that there was insufficient data in the literature to make recommendations regarding the timing of the initiation of VTE chemoprophylaxis [49, 50].

Traditional practice has been to delay VTE chemoprophylaxis in BSOI patients due to concern for exacerbation of bleeding. However, previous work suggests that the risk of failure of NOM due to hemorrhage caused from VTE chemoprophylaxis is exceedingly more rare than the thrombotic consequences of withholding said chemoprophylaxis. In a retrospective review of 312 BSOI patients, Eberle et al. found that only 12 patients (3.8%) failed NOM and of those, only one had been initiated on VTE chemoprophylaxis (which was initiated 3 days before the hemorrhagic complication) [51]. NOM failure concerns appear to be overestimated, with many groups finding no relationship between the timing of VTE chemoprophylaxis and transfusion requirement or the rates of NOM failure in BSOI patients [5, 12, 52–54]. Lack of NOM failure has

also been reported in trauma patients with BSOI and blunt cerebrovascular injury (BCVI) on therapeutic heparin infusion [55], further highlighting the safety of chemoprophylaxis dosing in BSOI patients. Interestingly, there are reports of *higher* rates of failure of NOM, as well as higher rates of VTE, in BSOI patients *not* on VTE chemoprophylaxis as compared to patients on chemoprophylaxis [17]. Ultimately, the literature reports that variables most predictive of failure of NOM in BSOI patients are multiple solid organ injuries, higher injury severity, nonliver injury (splenic or renal), positive abdominal ultrasonography findings in the ED, amount of free fluid on computed tomography >300 mL, admission lactate, and need for blood transfusion, *not* VTE chemoprophylaxis itself [4, 8]. The contention that VTE chemoprophylaxis using current dosing regimens increases bleeding risk in BSOI patients is simply not supported by data [12, 15–17, 53, 56].

The lack of evidence linking VTE chemoprophylaxis to increased bleeding risk in BSOI patients is likely related to (1) the fact that BSOI patients are almost all hypercoagulable soon after ICU admission and (2) current prophylaxis dosing is insufficient to render BSOI patients hypocoagulable. In a phase II randomized controlled trial comparing TEG-guided VTE chemoprophylaxis versus standard-of-care (timed chemoprophylaxis, not individualized) in 50 trauma patients, Harr et al. administered dalteparin on day one of ICU admission at a dose of 5000 IU if the TEG R_F (difference between the R time on kaolin TEG minus the R time on kaolin TEG with heparinase) was less than 1 minute and then increased dosage based on consequent R_F values for a goal R_F of 1.0–1.4 [57]. They ultimately found that anti-Xa levels remained below recommended prophylaxis values in both the control and TEG-guided chemoprophylaxis groups, and a decreased fibrinogen contribution to clot strength in the TEG-guided group (suggesting increasing doses of LMWH may reduce fibrinogen contribution to clot strength) [57]. Similarly, Van and colleagues performed citrated kaolin TEG and heparinase TEGs in 61 patients admitted to the surgical trauma ICU and found that R_F was near zero in patients who developed a DVT, indicating a subtherapeutic level of chemoprophylaxis [58]. These data suggest that, at best, current prophylaxis regimens are rendering BSOI patients less hypercoagulable as opposed to hypocoagulable.

Figure 35.3 illustrates the aforementioned concepts by demonstrating several theoretical scenarios for a BSOI patient. Scenario "A" represents traditional thinking, which is that VTE chemoprophylaxis transitions patients from a hyper- to a hypocoagulable state, thereby increasing the risk of bleeding. As mentioned, this logic is not supported by data. Scenario "B" represents the ideal state of transitioning a BSOI patient from hyper- to normal coagulation status, thereby mitigating VTE risk without increasing bleeding risk. Finally, scenario "C" depicts what is likely happening in reality with current regimens; profoundly hypercoagulable patients are rendered less hypercoagulable with chemoprophylaxis, without any discernable effect on bleeding risk.

When, then, is the optimal time to initiate VTE chemoprophylaxis in BSOI patients? General strategies include fixed timing of administration or timing individualized to a patient's real-time coagulation status. While some animal models of tissue injury and hemorrhagic shock

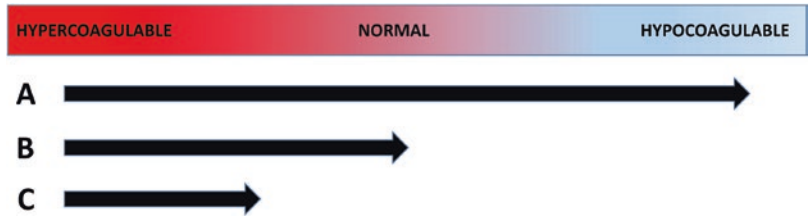


Fig. 35.3 Theoretical scenarios of hypercoagulability (x axis) and chemoprophylaxis strategies (black arrows) in BSOI patients, including Scenario "A" (traditional mindset), Scenario "B" (ideal), and Scenario "C" (current practice)

have identified hypercoagulability by TEG as early as 4 hours following injury [31], in severely injured humans, until recently, the majority of literature describes this transition to hypercoagulability occurs at 24–48 hours through retrospective data [18]. As such, Van and Schreiber recommend initiation of VTE chemoprophylaxis of BSOI patients at 48 hours [59]. Further, the Best Evidence Topic (BET) Reports reviewed all retrospective reviews examining VTE chemoprophylaxis in BSOI patients in 2018 and concluded that there is insufficient evidence assessing safety of low molecular weight heparin (LMWH) within 24 hours of trauma, acknowledging that retrospective studies suggest initiation of LMWH within 48 hours does not affect rate of nonoperative failure [60]. However, the latest research now highlights that hypercoagulability in BSOI occurs as early as upon admission to the ICU [16] and time to VTE may occur as early as within the first 72 hours [61], sooner than what was previously thought based on retrospective research. Based upon these data, we recommend initiation of chemoprophylaxis, specifically low molecular weight heparin, within 12 hours of injury.

Although adopting a practice of fixed, early initiation of VTE chemoprophylaxis is BSOI patients represents an improvement as compared to withholding it for days, such a strategy still fails to appreciate the heterogeneity of coagulation profiles in trauma patients. For this reason, the decision to initiate VTE chemoprophylaxis should ideally be based upon objective, dynamic measurements of each patient's coagulation status. Currently, TEG is the only whole-blood point-of-care clinical assay that can identify hypercoagulability, and several studies have demonstrated hypercoagulability as defined by TEG correlates with the likelihood of subsequent VTEs in severely injured patients [58, 62]. While there has been some work examining the role of thrombin generation in predicting thrombotic risk in trauma patients, the current FDA-approved assays are all plasma-based and are not widespread in current trauma diagnostics [63]. As described, Harr et al. describe the utilization of TEG for guiding chemoprophylaxis and decreased fibrinogen contribution to clot strength in the TEG-guided chemoprophylaxis patients [57]. Similarly, Van and colleagues describe utilization of TEG to identify patients with subtherapeutic levels of chemoprophylaxis [58]. Finally, our recent work identified markers of hypercoagulability as LY30 of <0.5% at 12 hours and MA of >65.8 at 48 hours, both of which predict thrombotic morbidity with high sensitivity and specificity [16]. These studies support individualized chemoprophylaxis strategies based on objective evidence of hypercoagulability (such as the use of TEG for identifying hypercoagulable patients at high risk of thrombotic morbidity) in contrast to standard universal timing of initiation. Additional research is needed to delineate the role of dynamic measurements of coagulation status in the decision to initiate VTE chemoprophylaxis of BSOI patients.

In conclusion, ideally, initiation of VTE chemoprophylaxis should be personalized by risk stratification with hematologic assays (an algorithm for such a strategy based upon our own institutional data [16] (Fig. 35.4). Practically, and in the absence of such assays, we recommend initiation of VTE chemoprophylaxis as soon as resuscitation is complete and ideally, within 12 hours of admission.

Future Directions

While current data report immediate and nearly pervasive hypercoagulability in BSOI, future prospective studies are needed to investigate safety of initiating VTE chemoprophylaxis at timepoints as early as upon ICU admission and/or within the first 24 hours. These studies should include objective measurements of coagulation status to monitor for treatment response to chemoprophylaxis and to identify patients at risk of thrombotic complications despite chemoprophylaxis. The latter group of patients highlights another area of future research, as the mechanisms behind BSOI-associated hypercoagulability and thrombotic complications despite chemoprophylaxis are poorly understood. Mechanistic work examining the hypercoagulability of BSOI specifically is important, as it may

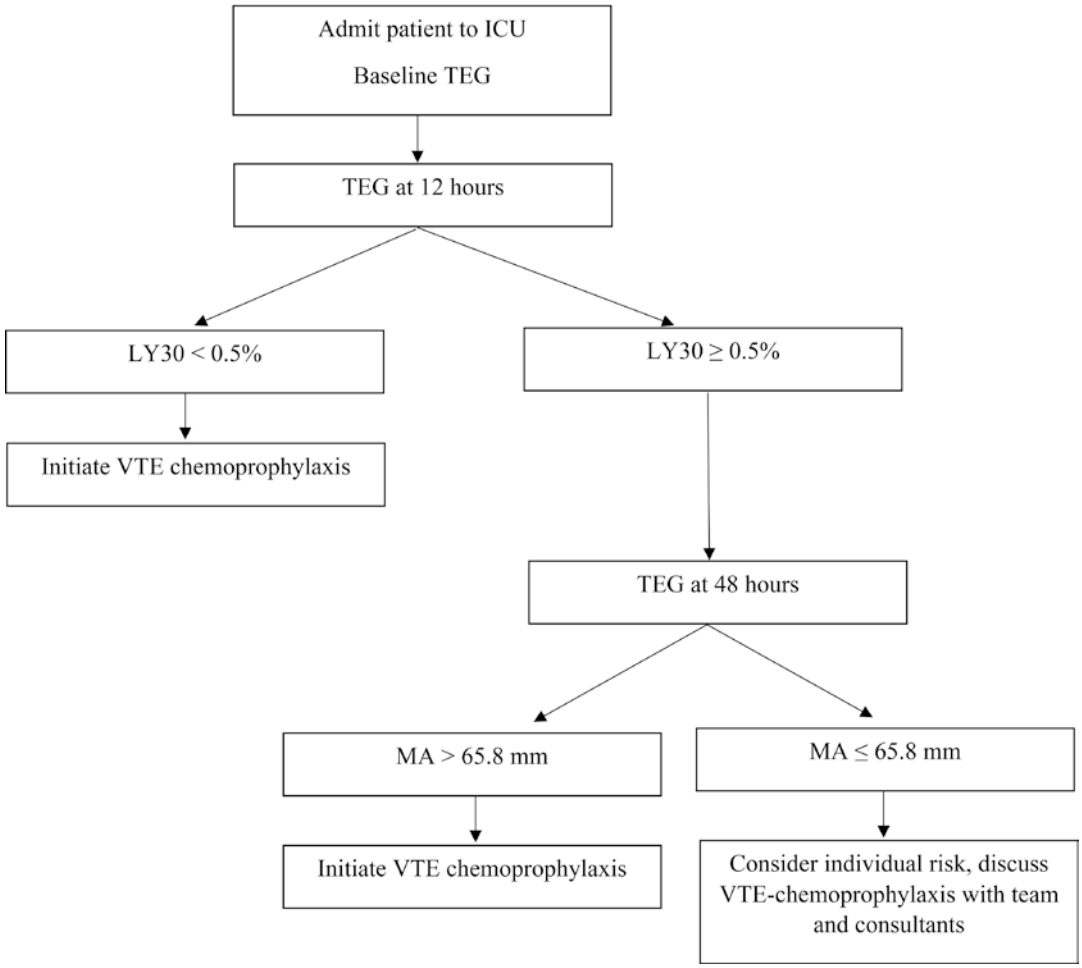


Fig. 35.4 Proposed decision algorithm for initiation of VTE chemoprophylaxis in blunt solid organ injury patients based upon serial thrombelastography

identify potential targets for intervention and individualized thrombotic risk mitigation. Future studies should also include investigation of alternative chemoprophylactic regimens, including those which incorporate platelet and fibrinolytic contribution to pathologic hypercoagulability.

Conclusion

BSOI patients are universally hypercoagulable upon ICU admission. This hypercoagulability manifests as shortened time to clot formation, increased clot propagation and strength and tPA resistance, and this profile persists for several days. What drives this hypercoagulable profile is unknown, but solid organ tissue injury may be associated with a unique profile of coagulation proteins that modify the hemostatic and fibrinolytic system, either through interactions of myosin with fibrin, increased exposure of tissue factors, or propagation of endotheliopathy. Despite this universal hypercoagulability of BSOI, VTE chemoprophylaxis is often delayed due to a presumed risk of failure of NOM. However, the literature would suggest that failure of NOM is exceedingly rare, even with doses of therapeutic anticoagulation, and when balancing failure of NOM against risk of thrombotic morbidity, early VTE chemoprophylaxis is

prudent. While there is provider hesitance to initiate chemoprophylaxis early, VTE chemoprophylaxis should be started within 12 hours of admission in BSOI patients, in particular those with objective evidence of hypercoagulability on TEG. Finally, future research is needed to clarify the role of dynamic assays of coagulation such as TEG in both timing and dosage of VTE chemoprophylaxis in BSOI patients.

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Pregnancy

36

Daniel Katz

Introduction

Trauma in pregnancy is a common condition which may impact pregnancy. Mechanism vary including motor vehicle collisions, accidents, domestic abuse, falls, burns and others. Pregnancy is a hypercoaguble state marked with several changes in different areas of coagulation. As such, management of pregnant patients who experience a trauma warrants further discussion. The following chapter is a summary of trauma in pregnancy, followed by physiologic changes in prengnacy, and ends on discussing the implications of pregnancy on trauma management.

Mechanisms of Injury, Implications, and Outcomes for the Parturient and Fetus

The leading nonobstetrical cause of maternal death in pregnancy is trauma [1]. Trauma impacts 1/12 of all pregnancies, and almost 1% of all trauma admissions are pregnant women [2]. The most common type of injury is blunt trauma, accounting for over 2/3 of the reported trauma [1]. Common mechanisms include motor vehicle collisions (MVCs), falls, and assault, with MVC being the most common and the most deadly with a mortality rate of 13.7% [3]. Studies examining mechanisms and outcomes of trauma during pregnancy are numerous and heterogeneous the details of which are beyond the scope of this chapter. There are, however, certain features of specific injuries that might impact triage or treatments of pregnant patients. Mechanisms and injuries that occur in close proximity to the uterus, i.e., pelvis and abdomen, often portend worse outcomes for the fetus. For example, the presence of a pelvic fracture is an independent risk for stillbirth independent of gestational age [4]. This was further demonstrated by Cannada et al. who noted a fetal death rate of 30% if the patients suffered either pelvic or acetabular fracture [5]. Likewise, severity of injury in an MVC is directly related to the usage of seatbelts, as well as whether or not frontal/lateral airbags were present [6, 7]. Gestational age at the time of injury will also directly impact risk to the fetus. For example, abdominal injuries in parturients less than 12 weeks gestation are less likely to cause fetal injury as the uterus is still within the confines of the bony pelvis [8]. As the pregnancy progresses abdominal viscera are pushed cephalad with the uterus mid abdomen. It is therefore not surprising that there is a very high fetal mortality rate for penetrating injuries (40–65%) in the third trimester [9]. Risk of fetal death is also directly related to the severity of maternal inju-

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Table 36.1 Trauma organ injury scale for the gravid uterus

Grade	Injury description
1	Contusion or hematoma without placental abruption
2	Superficial laceration <1 cm in depth or placental abruption <25%
3	Deep laceration 1 cm in depth in the second trimester or placental abruption 25% but <50%. Deep laceration in the third trimester
4	Laceration extending to the uterine artery. Deep laceration with 50% placental abruption
5	Uterine rupture in second or third trimesters. Complete placental abruption

ries as well as the American Association for the Surgery of Trauma Organ Injury Scale for the gravid uterus (See Table 36.1) [10, 11].

Likelihood of coagulopathy is not necessarily related to the mechanism of injury [12], it is directly related to the severity of injury as well as the incidence of hypothermia, acidosis, and hemodilution [13]. The exception to this would be injuries that result in significant placental abruption which can rapidly cause coagulopathy and are discussed in detail below.

Physiological Changes in Pregnancy

There are a number of changes that occur during gestation that have significant implications on the management of a pregnant patient who suffers a trauma. These changes can be due to the physical strain of the growing uterus, hormonal changes from the placenta and ovaries, or from other sources. These physiologic changes are necessary to support the metabolic needs of the fetus as well as prepare the mother for the blood loss associated with labor and delivery. A comprehensive description of these changes is beyond the scope of this chapter [14]. Changes that will impact management in trauma but not coagulation will be discussed briefly, while those that impact coagulation management will be discussed in detail. It should be noted that many of the changes are required to optimize the blood flow to the uterus which can increase to 700 ml–1000 ml/min at term gestation and lead to rapid exsanguination in a trauma [15].

Table 36.2 Physiological changes in pregnancy

Physiologic change	Change in pregnancy	Implications in management
Cardiac		
Blood volume	Increased 40%	Significant blood loss occurs prior to hypotension, early diagnosis of hypovolemia challenging
Cardiac output/heart rate	Increased 50%	
Hemoglobin	10–12 g/dL	Physiologic anemia
Respiratory		
Diaphragm	Pushed cephalad	Chest tubes inserted at higher levels
Functional residual Capacity	Decreased 20%	Rapid oxyhemoglobin desaturation with apnea
Oxygen consumption	Increased 20%	
pH	7.42–7.46	Baseline respiratory alkalosis with decrease bicarbonate reduced buffering capacity leading to acidosis in trauma
$PaCO_2$	28–32 mmHg	
$HCO3^-$	19–22 mEq/L	
Airway edema/tissue swelling	Increased each trimester	Difficult laryngoscopy
Other		
GFR/creatinine	Increased 50%/decreased	Altered drug metabolism, abnormal measurements overlooked as "normal"
Gravid uterus	Aortocaval compression	Large drop in the cardiac output when supine

Furthermore, although changes to coagulation accelerate in the third trimester of pregnancy, many of the other physiologic changes, such as left ventricular hypertrophy, changes to the heart rate/stroke volume, or decreases in systemic vascular resistance, occur in the first trimester [16, 17].

There are several physiologic changes that do not directly impact coagulation but are crucial to managing pregnant patients and are summarized in Table 36.2.

Changes in coagulation at term are numerous and are briefly summarized in Table 36.3.

The increase in plasma volume relative to platelet formation is thought to be the main cause of the decrease in platelet count [18], although an increased platelet turnover at the uteroplacental

Table 36.3 Physiological changes in hemostasis factors during normal pregnancy

Platelet count	↓
Fibrinogen, FVII, FVIII, FIX, VWF	↑↑↑
FII, FV, FX, FXI	=/↑/↓
FXIII	↓
AT, PC	=
PS	↓
rAPC	↑
t-PA	↓
PAI-1, PAI-2, TAFI	↑
DD, F1 + 2, FMC	↑

VWF von Willebrand factor, *AT* antithrombin, *PC* protein C, *PS* protein S, *rAPC* activated protein C resistance, *tPA* tissue plasminogen activator, *PAI-1* plasminogen activator inhibitor-1, *PAI-2* plasminogen activator inhibitor-2, *TAFI* thrombin activatable fibrinolysis inhibitor, *DD* D-dimer, *F1 + 2* prothrombin fragments 1 + 2, and *FMC* fibrin monomer complexes

bed has also been implicated [19]. This decrease in relative platelet count is thought to have no impact on general hemostasis. Levels of fibrinogen, factors VII, VIII, and IX [20], as well as von Willebrand factor rise significantly [21], whereas there is a slight decrease of FXIII activity [22]. There is conflicting data on changes in factors II, V, X, and XI; however, these variations are minor and do not likely carry clinical significance [20]. Protein C and antithrombin appear to be unaffected by gestation, while a significant free and total proteins S fall is observed [23]. Acquired activated protein C resistance has also been reported [24]. As to fibrinolysis, changes in proteins that regulate this system, i.e., tissue plasminogen activator, endothelial derived plasminogen activator inhibitor-1, placenta-derived plasminogen activator inhibitor-2, and thrombin activatable fibrinolysis inhibitor (TAFI), result in a state of decreased fibrinolytic activity [23]. This overall hypercoagulable state combined with physiologic fibrinolysis is also supported by the increase of the markers of activation of coagulation such as D-dimer [25], prothrombin fragments 1 + 2 [26], fibrin monomer complexes [27], as well as thrombin–antithrombin complexes [28]. Several global assays of hemostasis such as thrombin generation assays [27, 29], viscoelastic tests [30], and euglobulin clot lysis time [31] also suggest a biological hypercoagulable state throughout the pregnancy.

Table 36.4 Normal range of hemostasis assays in pregnancy

Coagulation test	Normal range	Term pregnancy range
Traditional coagulation tests		
Platelet count (×10³/μ)	150–400	146–429
Prothrombin time (s)	12.7–15.4	9.6–12.9
International normalized ratio	0.9–1.04	0.8–1.09
Activated partial thromboplastin time (s)	26.3–39.4	22.6–35.0
Fibrinogen (mg/dL)	211–496	301-–696
D-Dimer (mcg/mL)	0.22–0.74	0.13–1.7
Viscoelastic tests		
EXTEM clotting time (s)	38–79	43–69
EXTEM A10 (mm)	43–65	50–73
EXTEM maximum clot firmness (mm)	50–72	60–78
FIBTEM A5 (mm)	6–22	13–28
FIBTEM A10 (mm)	7–23	14–30
FIBTEM maximum clot firmness (mm)	9–25	16–34
Kaolin TEG R time (min)	4–8	1.0–13.0
Kaolin TEG maximum amplitude (mm)	54–72	64.6–86.2

TEG thromboelastogram

The impact on specific hemostasis assays is seen in Table 36.4.

The sum of these changes, although asymmetric, amalgamate to a doubling of the coagulation capacity as their nonpregnant counterparts. It is important to note that changes in coagulation begin in the first trimester and accelerate as the pregnancy reaches term gestation. Reference ranges and estimated lab values should not be interpreted in a vacuum and should begin by estimating what would be physiologically normal at that time period in the pregnancy.

Lastly, it should be noted that in addition to changes mediated by maternal physiology, the placenta is also contributory. For example, it is known to express plasminogen activator inhibitors 1 and 2, while also producing the tissue pathway factor inhibitor (TFPI) and thrombomodulin [32–34]. It is also a significant source of tissue factor (TF). The role of TF in the placenta is not fully understood. It has been hypothesized that TF plays a nonhemostatic role in the placenta and serves as a signal for angiogenesis and maintenance of blood vessels through a variety of cell

signaling mechanisms [35–37]. It also plays a vital role in hemostasis and pregnancy maintenance. Erlich et al. [38] developed a low-TF murine model and found a 42% incidence of mid-gestational hemorrhage as well as an 18% incidence of fatal postpartum hemorrhage. Placentas of the low-TF mice were noted to have numerous blood pools in the labyrinth although it is not known whether these pools were a failure of angiogenesis leading to necrosis or a failure of hemostasis. Although the role of TF in the placenta may not be clear, its abundance and potency as an instigator of coagulation has been demonstrated [39, 40]. Circulating quantities of TF in nonpregnant women approach 0.003 ng/mg of total protein in the blood. This number increases to 0.004 ng/mg of total protein in the pregnant patient. Concentrations in the myometrium increase to 7.40 (4.9–11.40) ng/mg and increase further in the placenta to 31.17 (25.01–37.27) ng/mg of total protein, akin to a 10,000 fold increase relative to the amount in serum circulation [39]. It is no surprise that when disruption of the uteroplacental bed occurs, consumptive coagulopathy develops rapidly.

Coagulation and Hemostasis Monitoring in the Pregnant Patient

Given the complexity of the coagulation changes in pregnancy which are compounded by trauma, coagulation monitoring is a cornerstone of patient management. As stated above, in order to monitor and manage coagulopathy in the pregnant trauma patient one must first take into account the physiological changes in pregnancy and utilize pregnant reference ranges; as interpreting values from "normal" ranges provided by laboratories will lead to mismanagement (See Table 36.4). Standard coagulation tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT) are often utilized; however, they are severely limited for several reasons. First, these tests are but one piece of the coagulation puzzle and focus solely on thrombin generation. They do not measure fibrin polymerization, complements of fibrinogen or platelets, nor provide an estimation of fibrinolysis which can be a cause of bleeding in this patient population [41–43]. PT and aPTT testing are also insensitive markers for blood loss and coagulopathy and can remain normal even after 4L of blood loss [44]. It is therefore not surprising that correlation coefficients for blood loss and PT/aPTT have been demonstrated to be unrelated or demonstrate poor correlation [41]. Furthermore, these tests are often not available at the point of care, with reported turnaround times between 45 and 90 minutes greatly limiting their applicability in trauma [45]. These tests can be supplemented by adding a platelet count or fibrinogen assay; however, these tests suffer from some of the same limitations. The relationship between platelet count and coagulopathy is nonlinear and poorly understood. Fibrinogen assessment has been shown to be a sensitive and specific marker for hemorrhage progression in obstetric hemorrhage and will be discussed in detail below; however, traditional Clauss assays are again limited by turnaround times and provide only a quantitative assessment of the fibrinogen complement [46].

Viscoelastic tests such as thromboelastography (TEG) and thromboelastometry (ROTEM) are whole blood hemostasis assays that provide data on the entire spectrum of coagulation [14]. Using cup and pin technology (TEG 5000, ROTEM Delta/Sigma) or rheometry (TEG 6s), a real time curve demonstrating clot formation, propagation, strength, and lysis is generated in real time. Information on the initial clot kinetics is available within 2–3 minutes [14]. Data on fibrinogen and platelet complements are available within 6–8 minutes [47]. There are correlations between viscoelastic parameters and certain traditional coagulation tests in pregnant patients. For example, the TEG functional fibrinogen assay demonstrates agreement with the traditional Clauss assay [47]. The FIBTEM A5 (amplitude of the FIBTEM tracing in mm at 5 minutes after clot formation) has also demonstrated good correlation with the Clauss assay, with a value of 12 mm correlating with a plasma fibrinogen level of around 2.2 g/L [44]. FIBTEM A5 has also been shown as a predictor for hemorrhage progression in postpartum hemorrhage and when under 10 mm is associated with prolonged

bleeds and ICU stay [44]. An in-depth review on viscoelastic testing is outside of the scope of this chapter; however, viscoelastic tests serve a major role in managing obstetric hemorrhage and should be utilized in the pregnant trauma patient whenever available. Studies examining management and outcomes when viscoelastic testing is utilized have demonstrated superiority in regards to blood product administration, ICU admission, hysterectomy rates, length of stay, as well as cost when compared to traditional strategies including shock packs and formulaic transfusion [45, 48–51]. Management strategies, cut-offs, and example algorithms are discussed below.

Finally, in the pregnant trauma patient transplacental hemorrhage can occur leading to isoimmunization with blood antigens such as the D antigen on the Rhesus protein complex (RhD) in mothers that are RhD negative with a fetus that is RhD positive [52]. The risk of isoimmunization is directly related to the volume of the fetal blood that mixes with the maternal blood and ranges from 20% to 40% risk at volumes greater than 5.0 mL and 60–80% at volumes over 30 mL [52, 53]. The Kleihauer-Betke test is used to identify fetal blood in maternal circulation after an event. Fetal hemoglobin is selected out on a smear after acid administration that destroys adult hemoglobin and is then stained. The ratio of fetal to maternal cells is then used to calculate the volume of fetal cells in maternal circulation. Treatment with Rh_0 immunoglobulin should be administered within 72 hours of exposure, with a dose titrated to the amount of the fetal blood mixed. A dose of 300 μg will neutralize 30 mL of fetal whole blood and may need to be repeated [52].

Transfusion Strategies and Hemostatic Agents in the Parturient

General Management

Obstetric trauma is a special situation that implicates two individuals: the mother and the fetus. Treatment and stability of the mother should be prioritized as this will also lead to the best outcome for the fetus [54]. A general management strategy is presented in Fig. 36.1.

Management outside of hemostasis and coagulation is outside of the scope of this chapter; however, a few principles deserve mention. In terms of radiologic tests, abdominal screening sonography for blunt abdominal trauma remains sensitive and specific even in pregnant patients, with a sensitivity of 80% and a specificity of 100% for detecting the necessity of major abdominal surgery [55]. In certain cases, computerized tomography scanning may be helpful, especially in cases of penetrating injuries to the back or flank and should be considered when indicated in spite of the radiation risk to the fetus. Diagnostic peritoneal lavage in an unstable patient can be safely performed in pregnancy in order to diagnose intraperitoneal and uterine hemorrhage [56, 57]. Patients in the second and third trimesters should be placed in left uterine displacement, or have the uterus manually displaced in order to prevent aortocaval compression and hypotension. Initial fluid resuscitation can be performed with either crystalloid or colloid based solutions. Studies comparing the two solution categories such as SAFE and CRISTAL have been equivocal, controversial, and likely should be interpreted as exploratory [58, 59]. None of these studies included pregnant patients further limiting the applicability. Other principles of trauma resuscitation including hypovolemic resuscitation, permissive hypotension, and damage control resuscitation [60, 61] can be applied to pregnant patients; however, it should be noted that the uterus does not have the capability to autoregulate the blood flow. Periods of hypotension which might preserve the cardiac/renal/neurologic function for the mother may not be tolerated by the fetus; however, treatment of the mother should take priority. Lastly, should the mother experience cardiovascular collapse all Advanced Cardiac Life Support (ACLS) measures should be taken with two additions. First, the patient should be kept supine to allow for chest compressions with an additional person providing manual left uterine displacement. Second, should the return of spontaneous circulation not be achieved by the fourth minute (end of the second cycle) then resuscitative hysterotomy

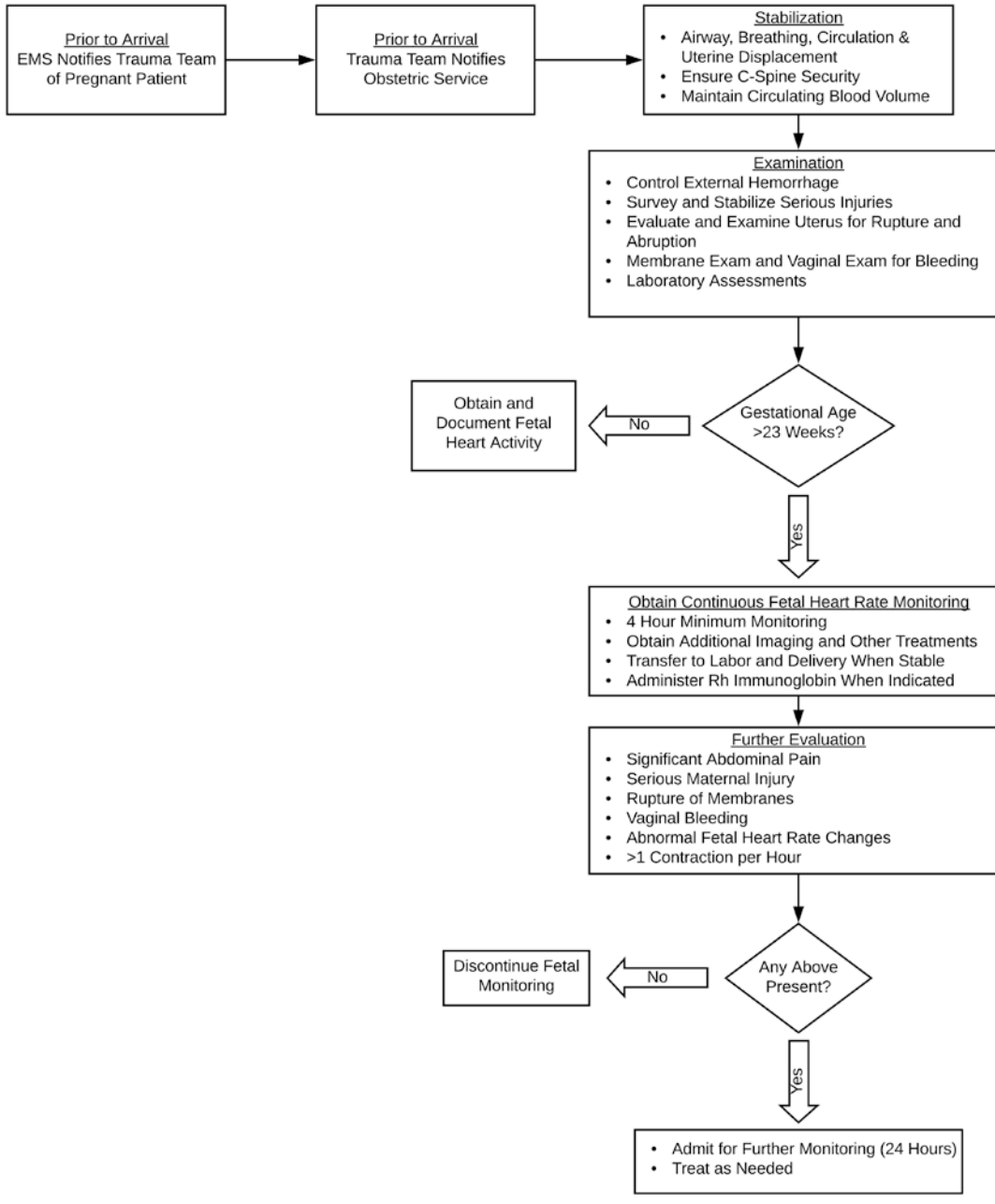


Fig. 36.1 General management strategy

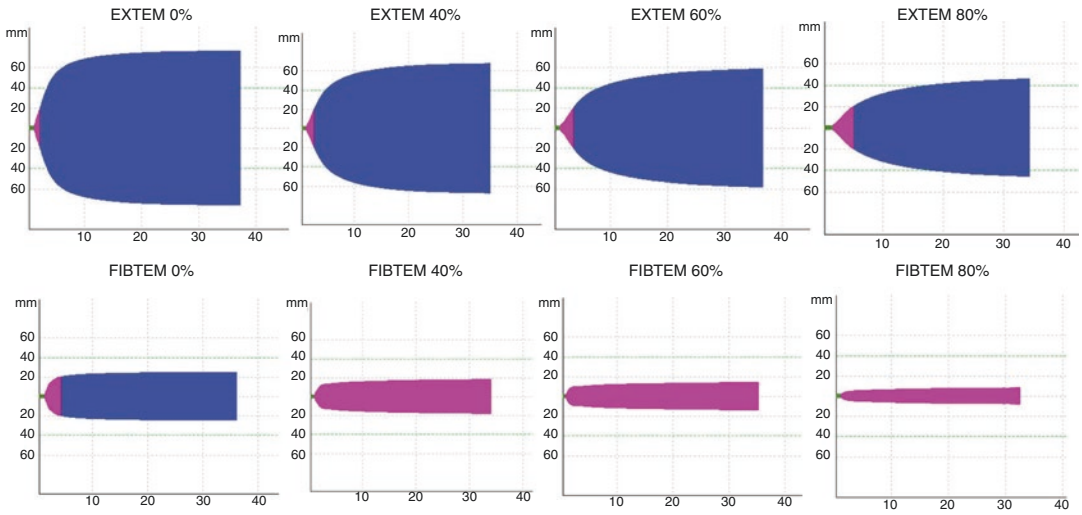


Fig. 36.2 Dilution of whole blood and the fibrinogen function in pregnancy

(formerly known as peri-mortem cesarean delivery) should be performed immediately at the location of the arrest with the goal of delivery of the fetus by the fifth minute [62, 63]. This should occur regardless of the viability of the fetus [63].

Initial Crystalloid Resuscitation and Impact on Coagulation

Prior to hospital arrival or in the early stages of trauma management, restoration of circulating volume is a priority. Common practice is to utilize crystalloid solutions to maintain circulation until an amount of blood loss occurs that warrants the replacement of blood components. As discussed above, the hypercoagulable state of pregnancy provides some protection against hemodilution in the early stages of traumatic bleed; however, in the event of active bleeding transitioning to blood component resuscitation should be considered early and is discussed below. The specific impact of crystalloids on coagulation is asymmetric, in that certain components are impacted earlier and more severely than others. An example is given from in vitro term pregnant patient below, who has had whole blood samples diluted with crystalloid at increasing percentages and examined using thromboelastometry testing (See Fig. 36.2).

In these tracings, the EXTEM and FIBTEM tests have both been activated with the tissue factor; however, a platelet inhibitor has been added to the FIBTEM test to specifically examine the contribution of fibrinogen to coagulation. In these tracings, the clotting time (CT) which is a representation of factor contributions remains normal until 60–70% hemodilution levels, demonstrating the resilience of this parameter to hemodilution. The fibrinogen component (FIBTEM) as measured by the amplitude at 5 minutes which is more predictive of the overall clot strength, however, begins to significantly decline much earlier around 35–45%. This reveals two important points in regard to the coagulation management in the pregnant trauma patient. The first is that traditional tests such as the aPTT, PT, and INR which measure factor contributions to coagulation will remain normal in pregnant patients until significant hemodilutions or derangements are present; likely long after intervention should have begun. The second is that even though the clot may begin to form, the clot strength is decreased which suggests there may be a role for early fibrinogen repletion in the pregnant trauma patient.

The Role of Fibrinogen in the Resuscitation of the Pregnant Trauma Patient

Fibrinogen serves many different roles in pregnancy. Outside of its role in coagulation, fibrinogen is critical for the development of maternal fetal circulation [64], supporting trophoblastic proliferation [65] and spreading in the early stages of pregnancy [66], as well as the development and maintenance of the placenta [67]. It is therefore no surprise that pregnant patients with fibrinogen disorders suffer from frequent miscarriage [68] and that the increase in fibrinogen in pregnancy is more pronounced that changes to other factors [14]. Fibrinogen has been shown to be an important predictor of outcomes in obstetric hemorrhage. Charbit et al. [69] examined coagulation profiles of over 100 pregnant patients with PPH and demonstrated that fibrinogen levels were independently associated with the severity of the hemorrhage. Every 1 g/L decrease in fibrinogen conferred a 2.6 increased odds of severe PPH. A presenting fibrinogen level ≤2 g/L had a positive predictive value of 100% for predicting hemorrhage severity. Since these works, other studies have examined using fibrinogen not only as a predictor of hemorrhage severity but also as a therapeutic target. McNamara et al. [70] recently reported 4 years' worth of clinical experience comparing a ROTEM algorithm based on early fibrinogen concentrate administration and demonstrated that when compared to formulaic transfusion early fibrinogen repletion led to a dramatic decrease in the units of blood products transfused 6 (3–12) vs. 3 (2–5) $p < 0.001$ as well as a decrease in the total volume of blood products needed (L) 1.7 (0.8–3.1) vs. 0.8 (0.6–1.6) $p < 0.001$. This translated to a significant decrease in the incidence of transfusion associated circulatory overload (TACO) as well as trends towards less massive red blood cell transfusions and ICU admissions. Other investigators have examined preemptive fibrinogen repletion early in PPH. In the OBS-2 study by Collins et al. [51] 55 pregnant patients with PPH to preemptive fibrinogen repletion vs. placebo and found no benefit to patients if their FIBTEM A5 was >12 mm, indicating that in the management of PPH the increase in fibrinogen appears to be a physiologic buffer. There are no data on the role of early fibrinogen repletion in pregnant trauma patients; however, in many circumstances data from PPH can likely be translated depending on the nature of the injury. In cases where the uterus is impacted and/or the fetus is delivered it is likely that the translation of this research is applicable. In cases where there is no trauma to the uteroplacental bed nor any evidence of obstetric hemorrhage care should be taken when applying these principles. More simply, childbirth and trauma are indeed distinct events that should be managed differently. Finally, it should be noted, however, that although the increase in fibrinogen may be a physiologic buffer in PPH, the increased levels may be required for placental maintenance which is not a concern in PPH as the baby is delivered, but may be critical to preventing an abruption in the pregnant trauma patient. More studies are needed in this area.

In regards to fibrinogen repletion, depending on the geographic location and the resource availability either fibrinogen concentrate or cryoprecipitate may be utilized. Fibrinogen concentrate is advantageous as it is easy to store, has a long shelf life, is virally inactivated, and has consistent fibrinogen concentration per dose relative to cryoprecipitate [71]. It is also available quickly after reconstitution, whereas a cryoprecipitate requires 20 minutes to thaw [72]. Reconstituting fibrinogen concentrate requires training as improper mixing can lead to foaming of the product which can lead to waste. Fibrinogen concentrate is costly depending on the location which has made utilization in the United States lag behind Europe [73]. The concentration of fibrinogen in fibrinogen concentrate is 20 g/L compared to 15–17 g/L in the cryoprecipitate [72]. Approximately, 4 g of fibrinogen is needed to raise the blood fibrinogen level by 1 g/L in a 70 kg patient. Although it would seem that fibrinogen concentrate has several advantages over a cryoprecipitate an advantage of a cryoprecipitate is that it contains FVIII, FXIII, von Willebrand factor, and fibronectin. In cases of extensive hem-

orrhage, repletion of these additional factors is necessary; however, data comparing a cryoprecipitate to a fibrinogen concentrate are equivocal and more data are needed [74]. Currently, the decision to use a fibrinogen concentrate or a cryoprecipitate should be determined by local factors. Nonetheless, repletion of fibrinogen in the pregnant trauma patient should be an early goal and should be an early component of any massive transfusion protocol for pregnant patients.

Formulaic Transfusion and Goal-Directed Therapy

The current guidelines for transfusion strategies and thresholds are based on evidence specific to the nonpregnant patient and often recommend formulaic transfusions of packed red blood cells (PRBC), fresh frozen plasma (FFP), platelets, and cryoprecipitate in fixed ratios in an attempt to mimic the whole blood [75]. Although the rationale for a fixed ratio strategy is useful for the prevention of coagulopathy in nonpregnant patients, care should be given before applying these guidelines to obstetric patients. In the event of massive hemorrhage (multiple blood volumes or extremely rapid bleeding), adherence to formulaic transfusion practices is an advisable strategy. However, if the blood loss is not catastrophic or exceedingly brisk, a more targeted approach may be superior [76]. As stated above, the maternal blood volume is increased at term, and the concentrations of coagulation factors is increased as well. As such, for noncatastrophic bleeds, providing a formulaic early transfusion strategy could incite a dilutional coagulopathy. For example, in the nonpregnant adult factor VIII levels are between 50% and 150% activity levels, whereas in term pregnancy are between 143% and 353% [77]. Therefore, before accounting for storage degradation and variance of factor levels in a unit of FFP, a pregnant patient would have to consume or dilute approximately 148% activity level of factor VIII before FFP transfusion would increase the blood concentration of factor VIII. To further complicate transfusion management, in conditions where blood loss is relatively minor, however, there is significant disruption of the uteroplacental bed and concomitant release of tissue factor, and a significant coagulopathy could develop at relatively small volumes of blood loss. In fact, in obstetric hemorrhage, the correlation between coagulopathy and blood loss continues to be weak [70]. Unless facing rapid exsanguination, it may be that a goal-directed approach using viscoelastic testing is superior for monitoring and correcting coagulopathy. In PPH management, several studies have demonstrated a variety of benefits of using goal-directed therapy instead of formulaic transfusion with shock packs or clinical judgement [49, 50, 78]. In nonpregnant trauma patients, Gonzalez et al. [76] randomized 111 patients to either goal-directed therapy with viscoelastic testing to traditional coagulation testing and demonstrated a survival benefit (36.4% vs.19.6%, $p = 0.049$). Of note, patients required a similar number of PRBC units; however, the goal-directed group received substantially less FFP. This data is in line with studies examining the benefits of viscoelastic testing which demonstrates that by eliminating unnecessary FFP transfusions, the outcomes improve [50, 79, 80]. An example of goal-directed algorithmic approach to coagulation correction in pregnancy is seen in Fig. 36.3.

Prothrombin Complex Concentrates and Recombinant Activated Factor VII

The data for proper dosing and safety of Prothrombin Complex Concentrates (PCCs) in the pregnant patient is extremely limited and has only been examined in the context of PPH [81]. Utilizing PCCs carry a major risk of thrombosis in this population, and clinical experience is limited to case reports in women who had extreme coagulation derangements or refractory hemorrhage despite resuscitation [82–84]. A potential advantage of PCCs is the standardization of their composition and activity, unlike FFP. Other advantages include storage at room temperature and shelf-lives of over 36 months, which can make them highly advantageous in areas with

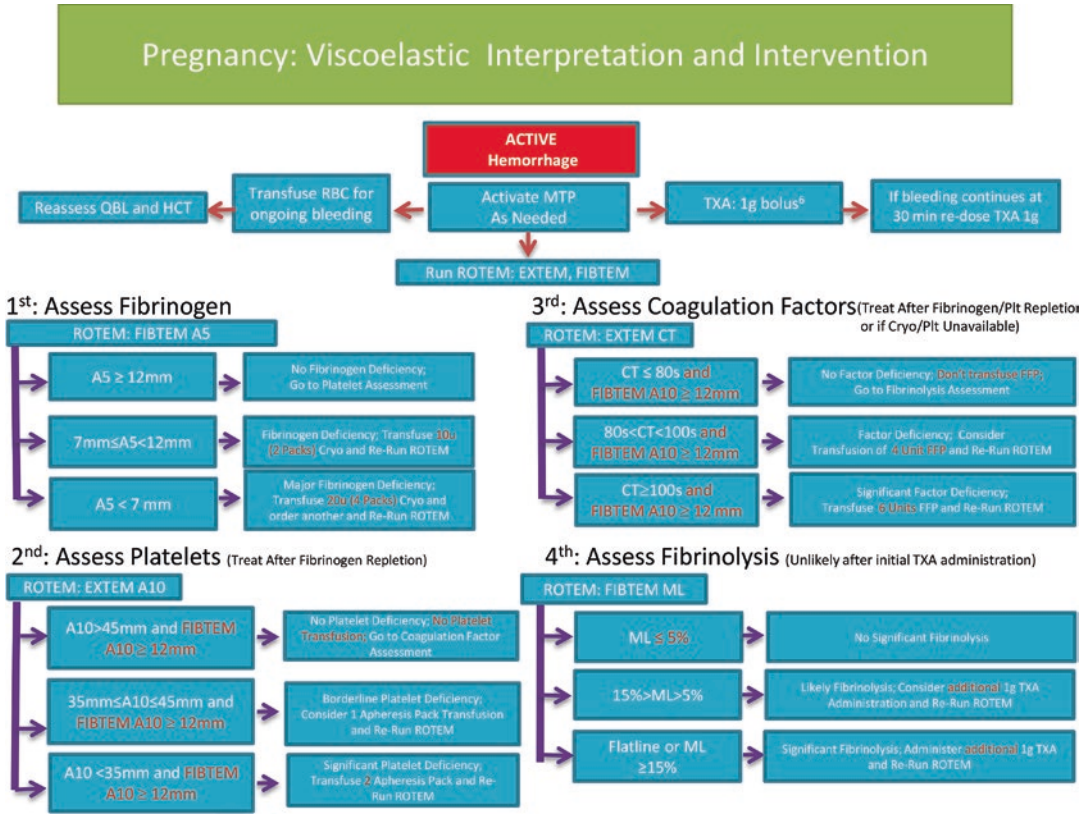


Fig. 36.3 Goal-directed treatment of bleeding in pregnancy – viscoelastic algorithm. QBL Quantitative blood loss, RBC Red blood cells, MTP Massive transfusion protocol, TXA Tranexamic acid, CT clotting time, A10 amplitude 10 minutes after clotting time, Cryo Cryoprecipitate, Plt platelets, FFP Fresh frozen plasma, LS Maximum lysis

limited blood bank resources. More data in the obstetric population are needed before they can be considered for anything other than severe life-threatening hemorrhages that are refractory to all other therapies [85]. Should PCCs be needed in this patient population, doses should be greatly reduced (5–10 u/kg) when compared to doses for acute vitamin k antagonist reversal (25–50 u/kg) [81, 86]. If available, viscoelastic testing should be used to guide therapy.

Similarly, if intractable life-threatening hemorrhage is encountered, recombinant activated factor VIIa (rFVIIa) may be considered. Similar to PCC, rFVIIa has been linked to both arterial and venous thromboses [87] and has no proven survival benefit [81]. There is no optimal dose range for the parturient, but 60–90 μg/kg IV is mentioned in guidelines [87]; however, lower doses (20–40 μg/kg) have been utilized and carry less risk of thrombosis and are likely a better starting point [88]. As with PCC, careful titration of the dose with repeated viscoelastic testing is advisable and has been used in the pregnant population with coagulation disorders in very small numbers without incident [89].

Antifibrinolytic Therapy

Tranexamic acid (TXA) binds to lysine residues in plasminogen preventing co-localization of plasminogen with tPA on fibrin, resulting in inhibition of fibrinolysis, which is believed to be a contributor to traumatic bleeding. Following the CRASH-2 trial [90], significant interest was generated in the utility of preemptive antifibrinolytic

therapy in trauma patients. Since that time, several guidelines have recommended the administration of TXA to trauma patients if it can be administered within the first 3 hours. Further, this study did not demonstrate an increase in thromboembolic complications which has been after administration in other studies [91]. However, none of the patients in the CRASH-2 trial were pregnant, and even if there was no increase in thromboembolism in this cohort, it could be a potential complication in a pregnant patient given the hypercoagulable state of pregnancy as discussed above. The largest study to date examining the utility of TXA in the pregnant patient was the World Maternal Antifibrinolytic Trial (WOMAN Trial) [92]. It included over 20,000 parturients across almost 200 hospitals in 21 countries. Women suffering from PPH were randomized to receive 1 g IV TXA after 500 mL of blood loss vs. placebo. Those who received TXA had a decreased incidence of death from bleeding (RR 0.64 [95%CI: 0.49–0.85 $p = 0.045$]). If the TXA was administered within 3 hours of delivery, the maternal death rate from bleeding was lowered from n(%) 127 (1.7) to 89 (1.2), a 30% decline. Like CRASH-2 the WOMAN trial found no increase in thromboembolic events in women who received TXA. However, the WOMAN trial had some of the same flaws as CRASH-2 in that many of the sites were low resource settings without access to viscoelastic testing, nor had the capability to detect nonlife threatening thromboembolic complications. However, given the randomized nature of these studies and the large number of patients, preemptive administration of TXA to the pregnant trauma patient within 3 hours of injury will likely yield more benefit than risk. Newer formulations of TXA that are more specific in their action at the site of injury are in development but will need to be validated in large studies before they are adopted in this patient population [93]. Lastly, a final point should be discussed prior to applying the principles of the WOMAN trial to the pregnant trauma patient. Currently, one of the proposed mechanisms by which hyperfibrinolysis may present itself in PPH is through an upregulation of fibrinolytic activity around the time of labor and delivery. This activity is required for proper separation between the uterus and the placenta. In the case of the pregnant trauma patient where there is no sign of abruption or the delivery of the fetus is not required, that stimulus may not be present and therefore the utility of TXA may be diminished or obliterated. As stated above, although there are parallels between trauma and PPH, childbirth in itself is a distinct event with specific changes in coagulation that may or may not apply in trauma depending on the circumstances.

Placental Abruption

In placental abruption DIC occurs due to the sudden release of procoagulant substances to the maternal circulation secondary to rupture of the uterine spiral arteries [94]. Moreover, the injured placenta and myometrium also release abundant tissue factors. As noted above, there is a 10,000 fold increase in the amount of TF present at the uteroplacental bed relative to the amount in serum circulation [39]. It is no surprise that when disruption of the uteroplacental bed occurs, consumptive coagulopathy develops rapidly. The reported incidence of placental abruption after trauma is high. In a population-based study of women hospitalized for injury in the Washington State from 1989 to 1997, nonseverely injured ($n = 266$) and severely injured women ($n = 28$) had an increased risk of placental abruption compared to noninjured pregnant women [95]. Likewise, in the aforementioned study of Schiff et al., women hospitalized for MVC had an increased adjusted relative risk of placental abruption as compared to pregnant women who were not hospitalized for a motor vehicle crash [96]. If placental abruption is suspected, a rapid assessment of fetal well-being and the need for immediate delivery are indicated. In the event of a large abruption coagulation assessment is warranted prior to utilizing neuraxial techniques of anesthesia. Once placental separation is complete and the initial coagulopathy is managed, the coagulopathy quickly corrects; however, patients may experience renal failure, sepsis, or other fatal complications secondary to the abruption [97].

Amniotic Fluid Embolism

Amniotic fluid embolism (AFE) is believed to occur when the fetal products of conception enter the maternal circulation including tissue factor [98], phosphatidylserine [99], endothelin 1 [100], arachidonic acid [101], and plasminogen activator inhibitor type-1 [102]. Although originally thought to cause circulatory collapse through RV outlet obstruction, it is now believed that presentation of RV dilation and failure may in fact be an immune-mediated phenomenon causing pulmonary vasocontriction resulting in acute pulmonary hypotension [103]. Similarly, the presence of fetal cells, amniotic debris, mucin, etc., in maternal circulation is not required to make the diagnosis and are often found in maternal circulation in normal pregnancy again pointing to a patient-mediated susceptibility to this condition [104]. It is not known whether the incidence or severity of this condition is related to the dose of fetal products encountered by the mother and the presentation of this condition is on a spectrum from a mild immune response to total circulatory collapse. Although not known to be a risk factor, trauma-associated AFE has been reported [105, 106]. MVC and improper seat belt use could be a risk factor for AFE [107]. Impact on coagulation is variable; however, the most common presentation is DIC. Treatment involves pulmonary vasodilators, circulatory support, and supportive measures. Both traditional component therapy and factor concentrates have been used to treat the DIC from AFE. A very small case series reported inferior outcomes in AFE patients who received activated factor VIIa; however, the data is of very poor quality, was likely more a marker of disease severity, and should not preclude the use of this agent if indicated [108].

Disposition Strategy and Venous Thromboembolism Prophylaxis

Once stabilized, pregnant patients should be risk stratified and placed in areas accustomed to managing pregnant patients if possible. Although an increasing number of obstetric ICUs have opened in the last several years, many institutions do not have the critical obstetrical volume to warrant a separate obstetrical ICU. However, hospitals and systems that have the medical specialties on site have created response teams and "virtual" ICUs to manage critically ill obstetric patients in a multidisciplinary manner [109, 110]. Disciplines involved in these units are often maternal-fetal medicine, neonatology, obstetric anesthesiology, cardiology, pulmonology, hematology, and surgery. A discussion on the critical care of obstetric patients is outside the scope of this chapter; however, one particular aspect of postevent care of this patient population deserves special consideration.

Venous thromboembolism (VTE) continues to be one of the main drivers of maternal mortality [111]. VTE can also be a complication of trauma in pregnant women. In a retrospective case-control study including 114 pregnant women admitted to a trauma service, 2.2% of women in the trauma group experienced venous thrombosis, whereas none were observed in the control group [112]. Similarly in a retrospective case-control study among 2795 women who sustained a fracture there was a 9.2-fold increased risk of thrombotic events. The decision on when and how to institute VTE prophylaxis should be individualized based on the risk profiles of the patient, the injuries, as well as the need for surgery. Appropriate dosing in this patient population is challenging, as both heparin and enoxaparin have relatively unstable pharmacokinetics in pregnant patients [113–115]. It is not uncommon to employ both mechanical and pharmacological prophylaxis in this patient population. In the event that patients require therapeutic levels of anticoagulation, frequent monitoring should be employed.

Finally, it should be noted that the hypercoagulable state of pregnancy ends quickly after delivery. Although some factors take days/weeks to reach their prepregnancy values, factors such as von Willebrand factor and factor VIII can normalize between 24 and 72 hours [116, 117]. Whether or not delivery of the fetus was required secondary to the trauma will therefore impact the coagulation management.

Summary

In summary, the management of pregnant trauma patients is complex. In addition to balancing the well-being of two patients at once, the anatomy and physiology that are encountered in nonpregnant patients are altered adding further complexity. Management strategies should focus on optimizing maternal oxygenation, ventilation, and perfusion which will lead to superior outcomes for the fetus. Pregnancy is a hypercoaguable state, with asymmetric changes in coagulation factors, fibrinogen, and fibrinolytic activity. Due to these circumstances, point of care testing should be utilized whenever possible using the principles of goal-directed therapy. Finally, coagulation management in pregnant trauma patients is extremely dependent on the circumstances of the need for delivery of the fetus or in the trauma that disrupts the uteroplacental bed. Trauma and childbirth are separate events and should be managed differently; however, depending on the trauma, they may need to be managed simultaneously.

A sample of term pregnant patient's whole blood successively diluted with a crystalloid solution at 0%, 40%, 60%, and 80% dilutions as demonstrated on EXTEM and FIBTEM testing.

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Liver Failure

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Introduction

Most coagulation factors, inhibitors, and fibrinolytic pathway proteins are synthesized in the liver parenchymal cells [1]. The hepatic cells dynamically respond to inflammatory stimuli, increasing the production of fibrinogen [2], plasminogen activator inhibitor (PAI)-1 [3], and thrombopoietin for platelet synthesis [4]. Acute or chronic liver failure can induce disturbances in coagulation and fibrinolytic pathways as well as in platelet production [5–7].

Coagulopathy due to liver failure has been traditionally viewed as a hemorrhagic disorder based on the prolongation of prothrombin time (PT) or international normalized ratio (INR). The evaluation of liver disease severity and the liver transplant allocation utilize the INR value to calculate the prognostic score [8]. Although PT/INR partially reflects synthetic function of the liver, prolonged PT/INR is rather a poor predictor of procedural bleeding risks [9, 10]. The functional state of coagulation is deemed rebalanced in liver failure because procoagulant activity tends to be maintained due to reduced coagulation inhibitors [11, 12]. The risk of bleeding can be heightened in patients with liver failure due to portal hypertension and development of esophageal varices [13]. Traumatic injury, invasive procedures, and major surgery can be a trigger which tips a fragile balance toward hemorrhage or thrombosis.

Clinical management of coagulopathy in liver failure has evolved in the recent years owing to biological, pharmacological, and mechanical interventions. Timely coagulation testing and more efficacious hemostatic and anticoagulant agents are being adopted instead of routine plasma transfusion according to PT/INR. This chapter will be focused on the pathophysiology, monitoring, and current therapies for liver failure-associated coagulation disorders.

Coagulopathy in Liver Failure – Pathomechanisms

Thrombocytopenia

Thrombocytopenia is frequently encountered in liver failure and is a frequent manifestation of chronic liver disease. Up to 50–70% of patients with cirrhosis or acute hepatitis with liver failure present with thrombocytopenia [14]. The severity of thrombocytopenia correlates with the extent of

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liver fibrosis in chronic hepatitis C [15]. Multiple mechanisms are involved in the pathogenesis of thrombocytopenia associated with liver failure. Thrombopoietin is the dominant hormone that regulates megakaryocytopoiesis [16], and its plasma level progressively decreases with the progression of cirrhosis [7]. Reduced platelet production can be caused by alpha-interferon [17], and it has an important implication in the treatment of hepatitis C [18]. Platelet sequestration in the enlarged spleen exacerbates thrombocytopenia frequently in the copresence of esophageal varices [19]. Immune-mediated platelet destruction and bone marrow suppression (viral or alcohol) are additional causes of thrombocytopenia in liver failure [20, 21]. Despite thrombocytopenia, there are some data indicating relatively normal platelet adhesion and aggregation in cirrhotic plasma due to elevated von Willebrand factor (VWF) [12, 22]. Notably, hemostatically most effective high-molecular VWF fractions are reduced in cirrhosis as shown in reduced in vitro platelet collagen binding. Therefore, sustained platelet adhesion is attributed to the reduced plasma level of ADAMTS13 (A disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13), a VWF-cleaving metalloproteinase [12, 23].

Taken together, platelet count is thus a useful laboratory test to assess the severity of liver failure and support the treatment of underlying conditions. However, it is difficult to determine the safe threshold of platelet count in liver failure to sustain hemostasis when elective or emergency invasive diagnostic or therapeutic procedures are necessary. The common threshold of $75 \times 10^3/\mu L$ is based on the retrospective studies which reported a higher incidence of bleeding after the liver biopsy when the baseline platelet counts were below 60–75 $\times$ $10^3/\mu L$ [9, 24]. However, some studies in laparoscopic surgery and transjugular liver biopsies suggest that a lower threshold, $50 \times 10^3/\mu L$, may be considered [25, 26]. For more invasive procedures including major trauma and surgical procedures, higher target platelet counts of $75–100 \times 10^3/\mu L$ should be aimed while bleeding persists.

Reduced Plasma Coagulation Factors and Inhibitors

Abnormal synthetic functions of the liver that manifest in prolonged PT/INR are regarded as an important prognosis marker of liver failure [8]. Reduced procoagulant protein levels of FII, FV, FVII, and FX are frequently detected by PT/INR in cirrhotic patients [11, 27, 28]. A major deficiency of routine clotting time tests such as PT and activated partial thromboplastin time (APTT) is that endogenous anticoagulant protein functions are not reflected in the results. Overall, thrombin generation (TG) in liver failure may remain in the normal range due to concomitantly reduced antithrombin (AT), protein C, and protein S [11, 12]. This state is often referred to as "rebalanced hemostasis" [1], which partly explains the poor correlation between prolonged PT/INR and clinical bleeding after minor procedures in liver failure [29, 30]. The fragile balance of coagulation is easily perturbed by hemodilution or exogenous anticoagulants, resulting in a bleeding tendency [31, 32] or thrombosis [33, 34]. An extracorporeal albumin dialysis, molecular adsorbent recirculation system (MARS), has been used for detoxification in patients with fulminant liver failure, or hepatorenal syndrome [35, 36]. Low AT activity in acute liver failure can contribute to thrombotic occlusions of the MARS circuit even when low-dose heparin is in use [37, 38].

Fibrin Polymerization Defects

Fibrinogen (Factor I) is synthesized in the liver and is the most abundant coagulation protein (1.5–3.5 g/L in normal plasma). It plays diverse physiological and pathophysiological roles in hemostasis, inflammation, wound healing, angiogenesis, and tumor growth [39, 40].

Abnormality of fibrinogen in liver failure is heterogeneous, and it can involve any step in the synthesis, metabolism, and breakdown of fibrin(ogen). Plasma fibrinogen level tends to be normal or elevated [41], but hypofibrinogenemia (<1.5 g/L) is not uncommon in late-stage

(Child-Pugh C) cirrhosis [42]. Fibrinogen is an acute phase reactant, and hyperfibrinogenemia is often encountered in inflammatory cholestatic diseases (primary biliary cirrhosis and primary sclerosing cholangitis) [43].

In the final stage of clot formation, fibrin monomers are polymerized by activated FXIII (FXIIIa) to stable cross-linked fibrin. Plasma FXIII activity is normal or mildly decreased in cirrhotic patients, but activity <50% may be encountered in the late stage (Child-Pugh C) cirrhosis [44, 45]. Low FXIII activity may be a prognostic marker for increased hemorrhagic and mortality risks. Poor fibrin polymerization due to hypofibrinogenemia or FXIII deficiency can be detected on fibrin-specific clot firmness using thromboelastometry [45].

Delayed fibrin polymerization can be caused by dysfibrinogenemia and may represent altered fibrinogen structures due to sialic acid maldistribution or excess [46]. Dysfibrinogenemia does not necessarily increase bleeding because fibrin clots tend to be more tightly packed than normally structured fibrin [47]. In the case of congenital dysfibrinogenemia, phenotypes are heterogeneous, and some may have bleeding symptoms (generally mild) while others have thrombophilia or remain asymptomatic [48]. Thrombophilia due to congenital dysfibrinogenemia is frequently due to impaired binding of plasminogen to fibrin and reduced sensitivity to tissue plasminogen activator (tPA) [48]. No standardized methods to assess a thrombophilic phenotype of dysfibrinogenemia, but structural evaluations of fibrin network, and tPA-induced fibrinolysis via viscoelastic coagulation tests may be potentially useful.

Hyperfibrinolysis

The liver synthesizes most of the proteins involved in fibrinolytic pathways. In liver failure, there may be an overall balance between simultaneously low pro- and antifibrinolytic proteins [49], but a profibrinolytic state can be found in some patients [50–52]. Tissue plasminogen activator (tPA) is constitutively excreted from the endothelial cells, and its plasma level can be increased by various signals including stress (vasopressin, epinephrine), inflammation (histamine, bradykinin), and coagulation (thrombin) [53]. Clearance of tPA is medicated by the liver [54], and it has been shown that plasma tPA levels increase during anhepatic phase of liver transplantation [55]. As an active enzyme, secreted tPA catalyzes plasminogen activation on the fibrin surface, and its activity is regulated by plasminogen activator inhibitor-1 (PAI-1). Decreased endogenous fibrinolysis inhibitors may lead to a profibrinolytic state. Low levels of α_2-plasmin inhibitor (antiplasmin) and FXIII activity render fibrin prone to plasmin-mediated degradation. Thrombin-activatable fibrinolysis inhibitor (TAFI) mediates cleavage of a lysine-binding site for plasminogen from the fibrin surface, and reduced TAFI activity in cirrhosis may increase fibrinolysis [50].

Coagulation Monitoring in Liver Failure

The use of PT/INR as a prognostic indicator is routine, but there are obvious limitations for their use in the setting of acute hemorrhage [56, 57]. PT/INR and aPTT are routinely run in plasma separated from the whole blood, and the typical turnaround time is in the range of 30–90 minutes [58]. The whole-blood point-of-care PT and fibrinogen tests reduce turnaround times [59, 60], but there is a paucity of clinical data in liver failure. When clotting time tests are ordered along with other routine coagulation tests such as platelet count or fibrinogen level, each test result is returned at different times. The provision of fragmented information makes it difficult to grasp the relative contribution of each abnormal segment to the entirety of coagulopathy.

In the perioperative setting, whole-blood viscoelastic coagulation testing has been considered as a practical alternative to a battery of routine coagulation tests. The early version of thrombelastography (TEG®; Haemonetics, Niles, IL) utilized a reusable metal cup and pin, and tests were performed in fresh whole blood without a coagulation activator. Although this approach was effective in the early days of liver transplantation with massive bleeding [28], the use of citrated whole blood, calcium chloride, and kaolin as an activator is preferred for improved

reproducibility in the current practice [61]. The interpretation of TEG® results can be subject to a degree of imprecision in choosing a specific therapeutic intervention. It is difficult to differentiate thrombocytopenia from hypofibrinogenemia using a single kaolin-TEG® test [62]. Perioperative assessment of fibrin-specific clot formation became popular since FIBTEM was introduced on the rotational thromboelastometry (ROTEM®; Instrumentation Laboratory, Bedford, MA) [63, 64]. A timely detection (10–15 minutes) of hypofibrinogenemia and/or hyperfibrinolysis allows targeted medical interventions to restore fibrin polymerization by replacing fibrinogen or administering an antifibrinolytic agent [65]. An abrupt loss of fibrin polymerization (flat-line tracing) has been associated with intracardiac and/or pulmonary thromboembolism [34], which seems to indicate that a fragile balance of coagulation can rapidly deteriorate into disseminated intravascular coagulation (DIC) in some patients with liver failure.

The choice of a coagulation activator potentially affects the interpretation of coagulation status (Fig. 37.1). Normal or high plasma FVIII levels tend to maintain contact-activated tests

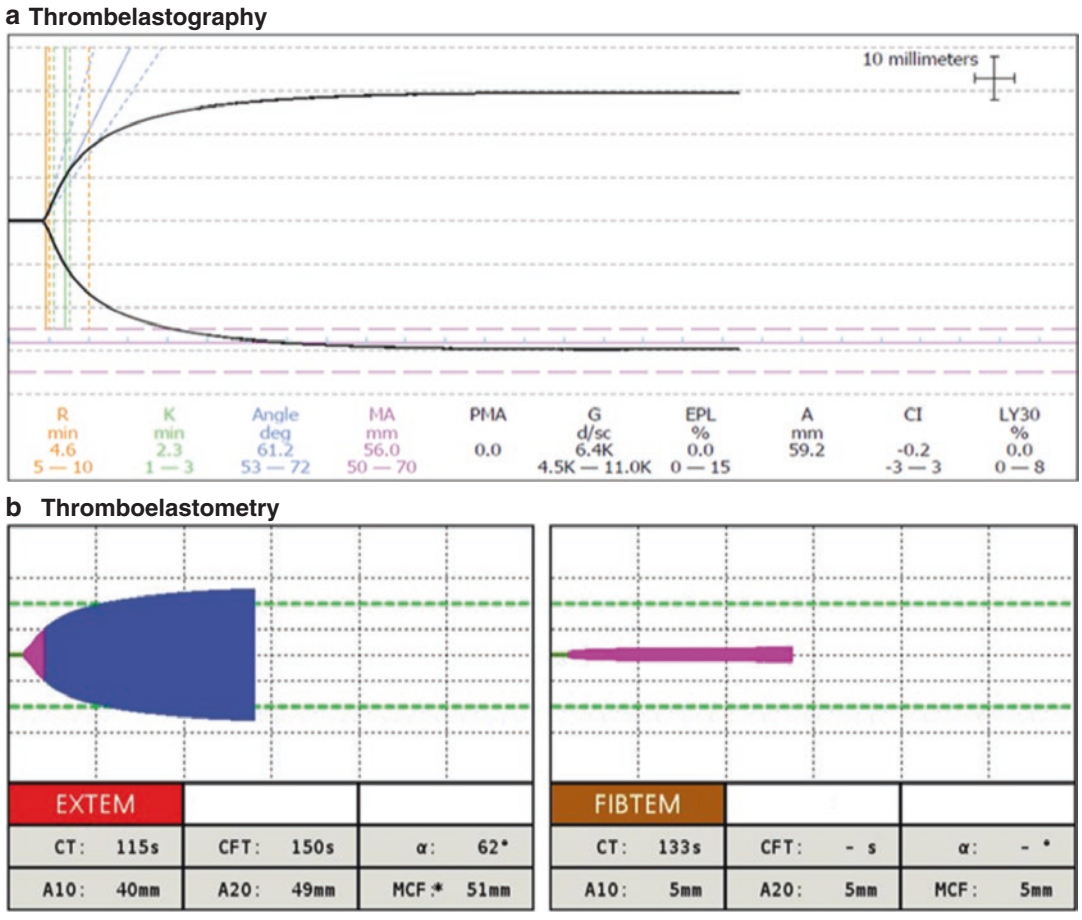


Fig. 37.1 Panel A. Kaolin-activated thrombelastography shows a short reaction time (R-time, 5–10 minutes), and reduced maximum amplitude (MA, 50–70 mm), suggesting only thrombocytopenia. Panel B. Tissue factor-activated thromboelastometry (EXTEM) shows prolonged clotting time (CT, 35–80 seconds), reduced A_{10} amplitude (43–65 mm) along with reduced FIBTEM A_{10} amplitude (9–24 mm). These results collectively indicate reduced vitamin K–dependent coagulation factors, thrombocytopenia, and hypofibrinogenemia. The parentheses indicate normal reference ranges

including APTT and kaolin-TEG® within normal limits unless bleeding or hemodilution becomes severe in cirrhotic patients [11, 41]. Exacerbations of coagulation factor deficiencies (FII, FV, FVII, and FX) can be more effectively detected using a tissue factor (TF)-activated test such as EXTEM on ROTEM® [66]. The detection of hyperfibrinolysis is also influenced by the choice of TEG® or ROTEM® tests. During liver transplantation, TF-activated FIBTEM followed by EXTEM was more sensitive to systemic fibrinolysis than kaolin-TEG® [51]. The varied sensitivities among different viscoelastic coagulation tests have important clinical implications because the timing of hemostatic intervention(s) can be significantly altered. The optimal intervention thresholds among TEG® and ROTEM® have not been established for patients with liver failure, but it is reasonable to choose specific targets at each institution based on the available hemostatic agents and clinical experience [67–70].

Testing platelet function remains controversial because thrombocytopenia is common in liver failure [14], and thrombocytopenia alone can affect platelet aggregation assays [71, 72]. Platelet count is tested in a static condition, and thus platelet function under flow conditions cannot be evaluated. As mentioned above, platelet adhesion/aggregation may be normal due to rebalanced VWF and ADAMTS-13 activities despite thrombocytopenia [12, 23]. Clot firmness of TEG® and ROTEM® are reduced in the presence of thrombocytopenia (50–75 × 10^3/μL) due to reduced interactions between thrombin-activated platelets and fibrin [63]. VWF activity and platelet adhesion cannot be assessed on these viscoelastic tests [73].

Hemostatic Interventions in Liver Failure

Treatments for Thrombocytopenia

Desmopressin (1-deamino-8-D-arginine vasopressin) is considered as an adjunct in the setting of platelet dysfunction. It activates vasopressin V_2 receptors on the vascular endothelial cells and induces the secretion of FVIII and VWF [74]. Prophylactic desmopressin treatment in liver resection lead to moderate increases in FVIII and VWF levels compared to placebo, but it fails to reduce allogeneic blood transfusion in cirrhotic or noncirrhotic patients [75]. Inefficacy of desmopressin may be explained by rebalanced VWF and ADAMTS-13 levels [12, 22].

Prophylactic platelet transfusion is tempting in thrombocytopenic patients with liver failure. However, the transfusion of stored platelet concentrates may not immediately improve either platelet count [76, 77] or platelet function [78, 79]. In the retrospective studies in liver transplantation, the exposure to platelet transfusion has been associated with lower graft survival and higher mortality [80, 81]. Room temperature storage of platelets presumably increases the risk of bacterial contamination and posttransfusion sepsis [81]. These risks may be mitigated by improved test methods to detect bacterial contamination [82], and pathogen reduction technologies applied to platelet units [83, 84]. However, noninfectious risks of platelet transfusion including acute lung injury, immune modulation, and thrombotic complications are possible [85, 86], Taken together, it is logical to consider platelet transfusion for active bleeding in the presence of severe thrombocytopenia (<50 × 10^3/μL), but there is a paucity of efficacy and safety data on platelet transfusion in liver failure.

Thrombopoietin Receptor Agonist

The potential use of an oral thrombopoietin receptor agonist was recently explored in a prospective randomized controlled trial in cirrhotic patients with thrombocytopenia (n = 292) before elective invasive procedures [87]. A 2-week course of eltrombopag (75 mg daily) increased platelet count to 100 × 10^3/μL from the baseline of 40 × 10^3/μL. The incidence of platelet transfusion was 28% with eltrombopag (n = 145) compared to 81% with the placebo (n = 147) (P < 0.001). The incidence of bleeding of World Health Organization (WHO) grade 2 or higher was also reduced in the eltrombopag group (17%) vs. the placebo group (23%). The study was terminated due to higher incidences of portal vein thrombosis with eltrombopag (6 cases vs. 1 with

placebo). The thrombotic events coincided with platelet counts that peaked over 200 × 10^3/μL within 2 weeks of the procedure (median, 8.5 days after the last eltrombopag dose) [87]. The interaction between preoperative thrombopoietin therapy and postoperative proinflammatory state warrants a future investigation.

Plasma Coagulation Factor Replacement

In the early days of liver transplantation, plasma transfusion was the mainstay intervention to correct major coagulopathy due to massive hemorrhage [88]. The changes in procoagulant factor levels after 2–3 units of plasma are rather small, minimally improving thrombin generation [31, 89], or fibrinogen level [90]. Larger amounts of plasma transfusion can be associated with transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO) [91, 92]. A paradoxical increase in bleeding after plasma transfusion can occur during liver surgery due to elevated central venous pressure [93, 94]. Some of the limitations of plasma transfusion can be overcome by the use of prothrombin complex concentrate (PCC).

PCC is a purified, lyophilized vitamin K-dependent (VKD) factor concentrate derived from the pooled plasma from human donors [95]. Commercial PCC products are categorized as either 3-factor (FII, FIX, and FX) or 4-factor PCC (FII, FVII, FIX, and FX). All PCC products undergo at least one form of pathogen reduction, and many undergo at least two steps [95]. Historically, 3-factor PCC was used to treat hemophilia B before purified FIX concentrate became available, and the PCC dose is standardized to the FIX content of approximately 500 international units (IU) per vial. The main indication of PCC today is for acute warfarin reversal using a 4-factor PCC product [96]. Higher concentrations of VKA factors (15–25 IU/ml) in reconstituted PCC compared to plasma (0.8–1.2 IU/ml) allow for rapid reversal of warfarin anticoagulation without circulatory overload [96, 97]. Several important differences in coagulation exist between warfarin anticoagulation and coagulopathy due to liver failure. Plasma AT activity is usually in the high normal range in warfarin-treated patients with high INR. Conversely, a high MELD score is associated with concomitant decreases in VKD factor levels and AT activity [31, 98]. The measurement of endogenous thrombin potential clearly demonstrates distinct thrombin generation (TG) kinetics at low AT activity in liver failure from those in warfarin anticoagulation (Fig. 37.2). The onset of TG is relatively well preserved in liver failure despite prolonged PT/

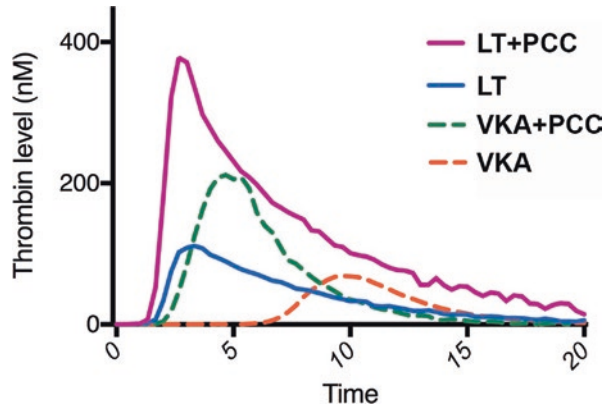


Fig. 37.2 Thrombin generation (TG) patterns in liver transplant (LT) and anticoagulation with vitamin K antagonist (VKA). Both samples had international normalized ratio (INR) over 2.0. The onset of TG is normal despite high INR due to low antithrombin (AT) activity to shut down activated factor X and thrombin. Addition of prothrombin complex concentrate (PCC) at 0.4 international unit (IU) per ml in vitro caused a much higher peak TG in VKA-treated plasma compared to LT plasma

INR values, reflecting critical AT deficiency (median, 25–29%). When 4-factor PCC was added ex vivo at 0.2 and 0.4 IU/mL (corresponding to 10 and 20 IU/kg), dose-dependent TG increases to PCC were observed, but the responses were more exaggerated in liver failure plasma than in warfarin-treated plasma [31]. Reduced requirement for PCC in liver failure is explained by reduced AT activity, which causes a delayed shut-down of procoagulant enzymes FXa and thrombin.

The use of 4-factor PCC has been reported in several case series of patients with acute liver failure suffering from active bleeding, undergoing invasive procedures, or liver transplantation [69, 99–101]. The median dose of PCC 25 IU/kg (~1500 IU) is most commonly used according to the baseline INR value [99, 100], or prolonged EXTEM clotting time [69]. The majority of patients required only a single dose of PCC [99, 100]. Thromboembolic complications associated with PCC include hepatic artery thrombosis, portal vein thrombosis, and myocardial ischemia. The reported incidences of thrombosis in the case series are 3–7% [69, 100, 101]. Repeated dosing should be cautioned because FX and prothrombin both have long half-lives (2–3 days) , and there is a link between the cumulative PCC dose and thrombotic events in liver failure [101]. Taken together, PCC is a potential alternative to plasma transfusion in bleeding associated with severe liver failure, but further clinical evaluations are needed for optimal dosing and safety data in this critically ill population.

Fibrinogen Replacement

Fibrinogen is an acute phase protein synthesized in the liver, and it is normally the most abundant plasma coagulation protein. Hyperfibrinogenemia is associated with a proinflammatory state in primary liver tumors, primary sclerosing cholangitis (PSC), and primary biliary cirrhosis (PBC) [43]. Hypofibrinogenemia (<1.5 g/L) may not be frequently observed until late-stage liver failure [42], but baseline fibrinogen levels can be quite variable, and a rapid deterioration of fibrin polymerization may be observed during hemorrhage and fluid resuscitation [41, 102]. Fresh frozen or thawed plasma is rarely indicated for fibrinogen replacement unless multifactorial coagulation deficiency and hypovolemia are evident. The adverse effects of plasma transfusion include volume overload, transfusion-related acute lung injury (TRALI), immunological reactions, and remote pathogen transmission risks [91, 92, 103]. Although cryoprecipitate has been the mainstay for fibrinogen supplementation in North America and United Kingdom [104], there are logistic issues including a need for blood typing, thawing, and short expiration (4–6 hours) after thawing. The hFC has several advantages as a source of fibrinogen including room temperature storage (2–25 °C for 60 months), rapid preparation (5–10 minutes), and blood-type free administration [104, 105]. There is not a universally established threshold level for fibrinogen replacement in liver transplantation or major surgery. Prophylactic use of hFC to achieve plasma level >2.0 g/L failed to reduce blood transfusion compared to the placebo during liver transplantation [106]. The available data suggest that it is reasonable to administer hFC to treat bleeding in the presence of fibrinogen level below 1.0–1.3 g/L or fibrin-specific clot firmness below 5–8 mm on ROTEM [64, 69, 70, 107].

Antifibrinolytic Therapy

The liver is the primary organ responsible for synthesizing and clearing most of the pro- and antifibrinolytic proteins. Primary fibrinolysis may occur during trauma and major surgery due to liver failure and the subsequent inability to clear tPA and plasmin. Conversely, secondary fibrinolysis in response to intravascular thrombus formation is possible in patients with reduced AT and other anticoagulant proteins [108]. Extremely severe coagulopathy signified by flat-line TEG® has been associated with fatal pulmonary thromboembolism during liver transplantation [33, 34]. Thrombolytic therapy with tPA or clot evacuation should be promptly applied in the case of intracardiac/pulmonary thrombosis [109]. More com-

monly, hyperfibrinolysis on TEG®/ROTEM® presents with microvascular bleeding accompanied with hypofibrinogenemia and thrombocytopenia. Either ε-amino caproic acid (EACA) or tranexamic acid (TXA) is effective in preventing plasmin activation, but multimodal interventions including fibrinogen replacement may be also required. Several retrospective studies showed no apparent increases in thromboembolic events, renal failure, or mortality associated with EACA or TXA to treat microvascular bleeding during liver transplantation [110, 111].

Transfusion Algorithm

Practice variability among surgeons and anesthesiologists can contribute to unnecessary blood component transfusions [93, 94]. There are currently three commercially available viscoelastic coagulation tests: ROTEM® Delta/Sigma (Instrumentation Laboratory, Bedford, MA), thrombelastography (TEG® 6S, Haemonetics, Niles, IL), and Quantra® (HemoSonics, Charlottesville, VA). Threshold values for hemostatic component interventions are different due to differences in activators or other reagents and varied measurement techniques [112, 113]. It is thus important to develop an institutional algorithm incorporating the existing guidelines and considering patient characteristics and specific clinical settings [69, 70, 107]. Table 37.1 summarizes a hemostatic intervention protocol based on EXTEM and FIBTEM on the ROTEM® Delta.

Table 37.1 Summary of hemostatic intervention protocol based on EXTEM and FIBTEM on the ROTEM® Delta

Agent	Diagnostics and decision points
DDAVP 20 μg	Administer prior to platelet transfusion if renal dysfunction or severe coagulopathy[a]
Platelet 1 U	FIBTEM-$A_{10} \geq 8$ mm and EXTEM-$A_{10} < 40$ mm
Platelet 1 U + Fibrinogen 2 g	FIBTEM-$A_{10} < 8$ mm and EXTEM-$A_{10} < 35$ mm
Fibrinogen 2 g	FIBTEM-$A_{10} < 8$ mm and EXTEM-$A_{10} \geq 35$ mm
TXA 1 g or EACA 5 g	Clot dissolution <20–40 minutes[b]
Plasma 10–15 ml/kg	EXTEM-CT > 90 seconds
PCC 10–15 IU/kg	EXTEM-CT > 90 seconds if volume load undesirable[c]
Protamine 25 mg	INTEM/HEPTEM-CT ratio > 1.0

Abbreviations: *CT* clotting time, *DDAVP* desmopressin acetate, *EACA* epsilon aminocaproic acid, *PCC* prothrombin complex concentrate, *TXA* tranexamic acid

[a]Coadministration of TXA or EACA should be considered as DDAVP releases tissue plasminogen activator

[b]Contraindicated in pulmonary thromboembolism or intracardiac thrombosis

[c]Contraindicated in disseminated intravascular coagulation, and coadministration of antithrombin may be considered if moderate to severe antithrombin deficiency due to acute liver failure

Thromboprophylaxis

Thromboprophylaxis for deep vein thrombosis (DVT) and pulmonary embolism (PE) is crucial in the postoperative management of trauma patients with liver injury. Venous thromboembolism (VTE) remains one of the highest causes of morbidity and mortality in trauma patients who survive the first 24 hours, and studies have reported a wide incidence of VTE ranging from 1% to 43% [114, 115]. A bundle of VTE prophylaxis should be considered, which includes both mechanical interventions, such as sequential compression devices, and pharmacological prophylaxis including unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). LMWH is typically reserved for those patients without concomitant renal dysfunction, but it has been shown to be more efficacious than UFH in VTE prophylaxis in the general trauma population [116]. There is appropriate concern for the use of UFH or LMWH in those with preexisting liver failure who have incurred traumatic liver injury. However, patients with cirrhosis have been found to be frequently hypercoagulable and are at increased risk for developing thrombosis. In these patients, the use of prophylactic LMWH has been shown to be safe although they have also been shown to have an increased response to LMWH [32, 117]. For chronic anticoagulation, the use of warfarin is difficult in patients with

liver dysfunction due to its narrow therapeutic window. Direct oral anticoagulants may be useful in this setting because their metabolims are only partially dependent on liver function (e.g., dabigatran ~20% hepatic elimination) [118].

Conclusion

The understanding of coagulopathy in liver failure has evolved as the rebalanced state of coagulation. Perioperative hemostatic therapies have also shifted from prophylactic plasma transfusion to targeted component-based interventions guided by point-of-care viscoelastic coagulation tests. Multimodal hemostatic interventions including factor concentrates may further reduce the requirement for allogeneic blood transfusion. Conversely, reduced anticoagulant levels in liver failure enhance the risk of thromboembolic complications, and further clinical evaluations are needed to optimize factor concentrate-based therapies.

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38

Pulmonary Coagulation in the Pathogenesis of Trauma-Associated Acute Respiratory Distress Syndrome

Christophe J. Langouët-Astrié and Eric P. Schmidt

ARDS in Trauma Patients

Acute respiratory distress syndrome (ARDS) is a complex, heterogeneous syndrome first described in 1967 by Ashbaugh and colleagues as a rapidly progressive respiratory failure arising from bilateral, noncardiogenic pulmonary edema [1]. Today, ARDS accounts for 10% of all ICU admissions worldwide and is associated with 35.3% ICU mortality [2]. ARDS can rise from various initial insults, either direct (i.e., pneumonia, aspiration of gastric contents, or pulmonary contusion) or indirect (i.e., sepsis or nonpulmonary trauma) to the lung [3]. The majority of ARDS cases worldwide are attributed to antecedent pneumonia (59.4%) or extrapulmonary sepsis (16%), with trauma only accounting for 4.2% of cases [2].

Unique respiratory physiological and clinical features have been identified for trauma-associated ARDS compared to nontrauma ARDS patients. Trauma-associated ARDS patients have lower mean respiratory rate and minute ventilation, and higher PaO_2/Fio_2 ratio and end-expiratory pressures, yet no difference in tidal volumes compared to nontrauma patients [3, 4]. Trauma-associated ARDS mortality is generally lower than that of nontrauma ARDS patients, potentially reflecting the propensity of trauma patients to be significantly younger, have fewer comorbidities, and have lower APACHE III scores [3, 4]. Interestingly, a review of the National Inpatient Sample (NIS), a database encompassing >69 million inpatient admissions in the United States between 2006 and 2014, demonstrated that in-hospital mortality declined for most ARDS causes over this period, potentially representing the impact of lung-protective supportive care such as low tidal volume ventilation [5]. In contrast, trauma-associated ARDS mortality rates increased during this study, potentially reflecting an increased attributable mortality of ARDS as more efficient emergency medical responses and resuscitation strategies improved early trauma survival. These trends suggest an emerging need to better understand trauma-associated ARDS.

Circulating biomarkers may provide insight into the etiology and localization of lung injury during ARDS. In differentiating between different ARDS risk factors, trauma patients were found to have lower (i.e., more normal) levels of intercellular adhesion molecule-1 (ICAM-1, an endothelial activation marker), surfactant protein D (an epithelial injury marker), von Willebrand

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factor antigen (an endothelial injury marker), and soluble tumor necrosis factor receptor 1 and IL-6 (inflammatory markers) when compared to nontrauma ARDS patients, after controlling for comorbidities [3]. These findings suggest that trauma-associated ARDS occurs with a relative paucity of alveolar endothelial, epithelial, and inflammatory injury in comparison to nontrauma ARDS. In contrast, trauma-associated ARDS is associated with equally abnormal plasma concentrations of circulating procoagulant and antifibrinolytic biomarkers as nontrauma ARDS, including the antifibrinolytic plasminogen activator inhibitor-1 (PAI-1) and the anticoagulant protein C [3]. These findings suggest a specific role for disordered systemic and intrapulmonary coagulation in the onset and progression of trauma-associated ARDS.

Pulmonary Coagulation After Nonpulmonary Trauma

In 1985, Jansson and colleagues observed that a gunshot-mediated femur fracture induced rapid deposition of circulating ^{125}I-labeled fibrinogen into the lungs of pigs, as assessed by an external gamma counter [6]. Similarly, systemic tissue factor release occurred in rats within 5 minutes of traumatic brain injury, leading to intrapulmonary microvascular coagulation and acute lung injury [7]. Activation of systemic hypercoagulation has been noted in other models of trauma, such as blast injury [8]. These preclinical observations of intrapulmonary coagulation after systemic injury have been validated by human studies demonstrating heightened systemic coagulation in multitrauma patients who progress to develop ARDS [9, 10]. Rapid posttraumatic activation of systemic (intravascular) coagulation has been attributed to activation of the extrinsic coagulation cascade (Fig. 38.1) by circulating procoagulant (tissue factor-enriched) microparticles [9, 11] released from the site of the initial injury [7]. This intravascular deposition of fibrinogen and fibrin within the lung after trauma is augmented by concurrent local, intraalveolar activation of coagulation [10, 12], attributed to release of tissue factor from the alveolar epithelium with subsequent airspace activation of the extrinsic coagulation cascade [13–16]. Indeed, the alveolar epithelium is the dominant source of alveolar tissue factor production [17], potentially reflecting a teleological role for alveolar coagulation in host defense.

Tissue factor-induced pulmonary fibrin deposition is augmented by trauma-associated impairment of endogenous anticoagulants and pulmonary fibrinolysis. Idell and colleagues performed bronchoalveolar lavages on 14 patients with ARDS (5 of whom were multitrauma patients) and 5 patients "at risk for ARDS" (all of whom were recovering from trauma) [18]. The investigators observed that ARDS patients not only demonstrated intraalveolar activation of the extrinsic coagulation pathway but also suppression of fibrinolysis despite sufficient availability of alveolar plasminogen. These findings indicated the presence of a fibrinolysis inhibitor, supported by Bertozzi and colleagues' study of patients with traumatic (n = 2) or nontraumatic (n = 6) ARDS. These investigators observed that alveolar fibrinolytics (specifically, urokinase) recovered from the bronchoalveolar lavage (BAL) of ARDS patients were nonfunctional by virtue of being completely complexed to PAI-1, an endogenous antifibrinolytic [19] expressed by the pulmonary epithelium [20], platelets [21], and other cell types. Interestingly, tissue plasminogen activator (tPA) was not recovered in BAL fluid from either normal or ARDS patients, potentially reflecting a relative absence of tPA expression in the pulmonary capillaries (as opposed to the endothelium of the bronchial circulation, [22]). The roles of other endogenous antifibrinolytics (e.g., thrombin-activated fibrinolysis inhibitor, TAFI) in the alveolar space after trauma are less well-understood although human studies of (nontrauma) interstitial lung diseases demonstrated increased BAL concentrations of TAFI [23].

Impairment of fibrinolysis has also been observed to occur within the intravascular space after trauma. Gando and colleagues measured

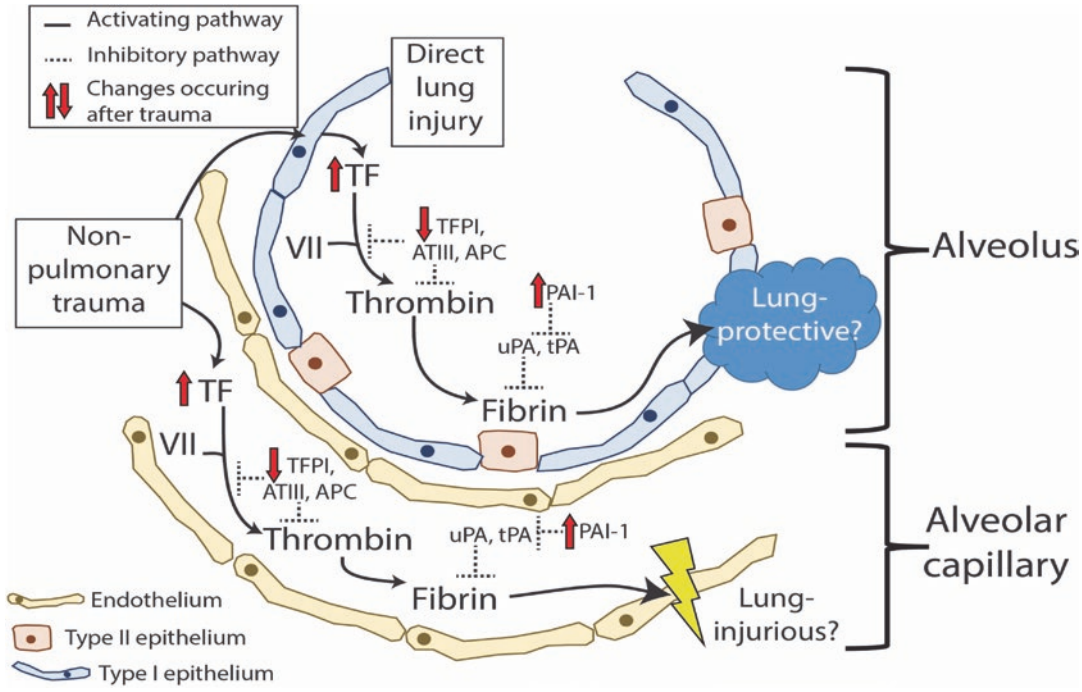


Fig. 38.1 Activation of pulmonary coagulation during trauma. Nonpulmonary trauma triggers release of circulating tissue factor and induction of airspace tissue factor. Direct lung injury similarly induces release of airspace tissue factor. Tissue factor activates the extrinsic coagulation cascade within both intravascular and intraalveolar spaces. Trauma/critical illness induces tissue factor release and dysregulation of endogenous modifiers of coagulation, such as loss of tissue factor pathway inhibitor (TFPI), antithrombin-III (ATIII), and activated protein C. In addition, there is shedding of cell-surface thrombomodulin and heparan sulfate (not pictured). Trauma/critical illness is additionally associated with upregulation of antifibrinolytics such as plasminogen activator inhibitor-1 (PAI-1), leading to suppression of urokinase (uPA) and tissue plasminogen activator (tPA)-mediated fibrinolysis. Extensive preclinical mechanistic studies of tissue factor suggest that coagulation within the airspace is host-protective, while intravascular coagulation is proinjurious. This compartmentalization, however, has not been clearly validated in human studies

plasma indices of coagulation in 41 trauma patients, 5 of whom had ARDS. These patients demonstrated both intravascular coagulation and induction of circulating PAI-1 [24].

This multifaceted activation of the extrinsic coagulation cascade (and inhibition of fibrinolysis) within the lung has been reviewed in detail by others [25–28] and is summarized in a simplified schema in Fig. 38.1. In this brief review, we will focus on the impact of the extrinsic coagulation cascade on the development of ARDS after trauma, with the goal to identify critical knowledge gaps that may explain the failures of coagulation-targeted therapies in both traumatic and nontraumatic critical illness.

Potential Contribution of Pulmonary Coagulation to ARDS Pathogenesis

ARDS is defined by the presence of diffuse alveolar damage, a histologic pattern characterized by alveolar fibrin deposition [29]. This deposition, presumptively occurring within both intravascular and intraalveolar spaces, is postulated to contribute to ARDS pathogenesis via multiple mechanisms:

- *Intravascular coagulation*, via production of microthrombi (either in situ or embolic to the lung from the periphery), impacts pulmonary

function via induction of physiologic dead space. Furthermore, thrombin and fibrin can directly activate platelets and endothelial cells, inducing vascular hyperpermeability. The pulmonary endothelial consequences of intravascular clot have been comprehensively reviewed by Evans and Zhao [30].

- *Intraalveolar coagulation*, via the production of alveolar fibrin, may directly inactivate surfactant [31], potentially leading to atelectasis and shunt physiology.
- Both *intraalveolar* and *intravascular coagulation* may promote lung injury via multifaceted interactions with regulatory pathways of lung inflammation, cell death, and repair. Protease-activated receptors (PARs) are cell-surface receptors activated by thrombin (PAR1, 3, and 4) or activated factor VII (PAR2) [26, 32]. PAR activation interfaces with inflammation and angiogenic pathways, promoting endothelial barrier dysfunction [25, 26, 28]. Accordingly, activation of PAR1 promotes the development of acute lung injury (via a TGF-beta dependent pathway) [33]. However, PAR signaling in ARDS pathogenesis is complex, with preclinical evidence suggesting that these receptors are dispensable for lung injury. PAR2 knockout mice were not protected from direct lung injury [34], and no differences in the severity of indirect lung injury were observed in PAR1, 2, 4, 1-2, or 2-4 knockout mice after high-dose intraperitoneal lipopolysaccharide [35].

  Similar to thrombin-mediated PAR activation, fibrinolytic enzymes may also impact alveolar function independently of their action on fibrin. Urokinase, a profibrinolytic expressed by the pulmonary capillary endothelium [36], has been demonstrated to impact epithelial apoptosis and repair via P53 signaling [20, 37, 38].

  Furthermore, fibrinolytic pathways may shape pulmonary fibrogenesis by influencing epithelial-mesenchymal transition [39].

Contribution of Key Components of the Extrinsic Coagulation Cascade to Trauma-Associated ARDS

As described above, posttraumatic pulmonary activation of the extrinsic coagulation cascade would be expected to promote alveolar inflammation, leading to and exacerbating trauma-associated ARDS. However, the signaling consequences of the extrinsic coagulation cascade are myriad, demonstrated by conflicting findings from investigations of three major "nodes" in this pathway: tissue factor activation, endogenous anticoagulants, and fibrinolysis.

- *Tissue factor-mediated activation of coagulation*. As detailed previously, multiple preclinical models of trauma demonstrate rapid induction of coagulation within the lungs, promoting the development of lung injury. Yasui and colleagues demonstrated that rats subjected to traumatic brain injury (TBI) immediately showered tissue-factor-enriched microparticles into the blood, which then accumulated within the lung. This pulmonary accumulation was injurious, as a direct thrombin inhibitor prevented post-TBI lung injury [7]. Tissue factor has been similarly implicated in observational clinical studies of trauma-associated ARDS. Trauma patients who developed ARDS displayed persistence of circulating tissue factor in comparison to clearance of circulating tissue factor in those who did not progress to lung injury [9]. While critically ill patients (traumatic and nontraumatic) simultaneously increase plasma expression of tissue factor pathway inhibitor (TFPI), this compensatory mechanism was found to be insufficient to prevent systemic hypercoagulation [40–42].

  While the release of circulating and alveolar tissue factor after trauma has been repeatedly described, the impact of pulmonary tissue

factor on ARDS pathogenesis is complex. As described in section "Potential Contribution of Pulmonary Coagulation to ARDS Pathogenesis," pulmonary tissue activation would be expected to induce the injurious consequences of intravascular and intraalveolar fibrin deposition. In support of this hypothesis, intravenous tissue factor blockade administered to baboons either before [43] or after [44] sepsis improved organ function. Similar pretreatment and posttreatment efficacy of intravenous tissue factor inhibition was observed in endotoxemic rats [45]. Conversely, whole-body [46, 47] or alveolar epithelial-specific [17, 46] deficiency of tissue factor worsened lung injury after a direct (intraalveolar) pulmonary insult. There was similarly no benefit of whole-body fibrinogen deficiency in a mouse model of acid-aspiration-induced pneumonia [48]. These discrepant findings could suggest a compartmentalization phenomenon in which intraalveolar (i.e., extravascular) tissue factor is protective against alveolar epithelial insults, while intravascular tissue factor is injurious after pulmonary endothelial insults (Fig. 38.1). Indeed, airspace tissue factor activation may be host-protective against infection by preventing systemic distribution of a pulmonary pathogen [49]. Furthermore, clots are a rich source of growth factors that may promote tissue repair [30]. Interestingly, myeloid or endothelial-specific tissue factor knockouts had no impact on multiple models of lung injury [17, 46, 50].

- *Impairment of endogenous anticoagulants*. The coagulation cascade is modulated by the expression and release of endogenous anticoagulants. TFPI, released from endothelial cells in response to thrombin stimulation [51], functions as an endogenous regulator of tissue factor-mediated activation of the extrinsic coagulation cascade. Endothelial surface (or circulating shed) heparan sulfates can potentiate antithrombin III in a heparin-like fashion, suppressing thrombin activity [52, 53]. Endothelial cell-surface thrombomodulin directly inactivates thrombin while promoting activation of protein C, an endogenous anticoagulant. Observation human studies have demonstrated that trauma-associated loss of these anticoagulants (e.g., circulating antithrombin III, [54]) predicts progression to ARDS. Similarly, loss of circulating antithrombin III in trauma patients [55] or activated protein C in nontrauma patients [56] is associated with ARDS mortality.

  Interestingly, trauma (and other critical illnesses) may promote coagulation not only by downregulating endogenous anticoagulants but also by altering their biological compartmentalization. Heparan sulfate and thrombomodulin are components of the endothelial glycocalyx, a glycan-enriched, gel-like layer that lines the endothelial surface in all blood vessels [57]. Localization of endogenous anticoagulants within the glycocalyx contributes to the nonthrombogenic nature of the endothelial surface [52]. Trauma, sepsis, and other critical illnesses induce glycocalyx shedding, leading to persistent elevations of plasma heparan sulfate and thrombomodulin fragments [53, 56, 58] that correlate with ARDS mortality [59]. Shedding of these cell-surface anticoagulants promotes circulating anticoagulation [53, 60, 61] while potentially (and paradoxically) inducing a procoagulant state on the denuded endothelial surface [62]. Similar decompartmentalization of endogenous anticoagulants has been seen in the airspace as ARDS is associated with shedding of heparan sulfate [63] and thrombomodulin [56] into the alveolar space.
- *Fibrinolysis shut down*. Observational studies have demonstrated that impaired fibrinolysis, mediated by either loss of plasminogen or, more frequently, increased expression of fibrinolysis inhibitors (e.g., PAI-1), occurs in trauma patients and is associated with ARDS onset and mortality [18, 24, 54, 55, 64]. Such loss of fibrinolytic activity occurs in both intravascular [24, 54, 55] and intraalveolar [18] spaces.

  As with tissue factor, however, the presence and impact of pulmonary fibrinolysis inhibition is complex. Upregulation of PAI-1

and other urokinase inhibitors may be offset by alveolar expression of α2-macroglobulin, which shields urokinase from large-weight inhibitors while maintaining urokinase amidolytic activity [65]. However, α2-macroglobulin binding inhibits nonfibrinolytic functions of urokinase, as complexed urokinase is unable to activate epithelial cells [65]. Furthermore, a murine acid aspiration model was unaffected by whole-body deficiency of PAI-1, suggesting that inhibition of fibrinolysis is dispensable for direct lung injury [48].

Attempts to Therapeutically Target Pulmonary Coagulation Abnormalities in Patients with Critical Illness

Given the potential contributions of tissue factor activation, loss of endogenous anticoagulants, and fibrinolysis inhibition to trauma- and nontrauma-associated ARDS, there is increasing interest in pulmonary coagulation as a therapeutic target in lung injury. Despite promising preclinical studies, human trials of coagulation-targeted therapeutics have been largely disappointing (Table 38.1). Notably, these studies have primarily enrolled nontrauma critically ill patients, reflecting reluctance to expose trauma subjects to potential hemorrhagic complications.

- *Tissue factor inhibitors***.** As described in section "Contribution of Key Components of the Extrinsic Coagulation Cascade to Trauma-Associated ARDS" of this chapter, preclinical studies have suggested a beneficial effect of inhibiting intravascular (but not intraalveolar) tissue factor. Disappointingly, clinical trials revealed no impact of intravenous administration of tissue factor inhibition on patient outcomes in sepsis (a cause of indirect lung injury, [66]) or severe community-acquired pneumonia (a cause of direct lung injury, [67]) (Table 38.1).
- *Administration of exogenous anticoagulants***.** Despite evidence of preclinical effectiveness

Table 38.1 Randomized, controlled, multicenter studies of coagulation-targeted therapies in critically ill patients with ARDS or illnesses predisposing for ARDS

Study	Study population/intervention	Impact on coagulation	Impact on inflammation	Impact on clinical outcomes
Tissue factor pathway inhibitor (TFPI)				
Abraham et al. [66]	1754 adult patients with sepsis, organ failure, and international normalized ration (INR) ≥1.2. 201 patients with sepsis, organ failure, and INR <1.2. Randomized to receive intravenous (iv) tifacogin (recombinant TFPI) 0.025 mg/kg/h or placebo × 96 hours. 35% of patients had undergone major surgical procedure in 7 days prior to randomization.	Increased circulating TFPI. Decreased plasma prothrombin fragment 1.2 and thrombin: antithrombin complex levels.	Not reported.	No change in 28 day mortality. No benefit in patients with P/F ratio <300 at study entry. Trend ($p = 0.051$) towardmortality benefit in patients with INR <1.2.
Wunderink et al. [67]	2138 adult patients (168 centers) with severe community-acquired pneumonia. TBI (within previous month) patients excluded. Patients randomized to iv TFPI 0.025 mg/kg/h, 0.075 mg/kg/h, or placebo × 96 hours.	Decreased plasma prothrombin fragment 1.2 and thrombin: antithrombin complex levels.	Not reported.	No change in 28 day mortality. No change in progression to ARDS. No change in pulmonary dysfunction.

Table 38.1 (continued)

Study	Study population/intervention	Impact on coagulation	Impact on inflammation	Impact on clinical outcomes
Antithrombin III				
Warren et al. [70]	3314 adult patients (211 centers across 19 countries) with sepsis/septic shock. 46% of patients were surgical. Excluded patients with severe cranial or spinal trauma in previous year. Randomized to intravenous antithrombin III (30,000 units over 4 days) or 1% albumin as control.	Increased circulating antithrombin-III levels.	Not reported.	No change in 28 day mortality in overall population or surgical subgroup.
Activated Protein C				
Bernard et al. [73]	1690 adult patients (164 centers across 11 countries) with sepsis and organ failure. 3.3% of experimental and 5.1% of control group had recent trauma. Excluded patients with TBI or "trauma considered to increase the risk of bleeding." Randomized to iv activated protein C 24 μg/kg/h vs. placebo × 96 hours.	Decreased plasma D-dimer.	Decreased plasma interleukin 6 (IL-6).	Decreased 28-day mortality (absolute risk reduction 6.1%).
Liu et al. [74]	75 adult patients (8 centers) with ARDS; patients with sepsis and APACHE-II >25 excluded. Excluded 4.1% of screened patients for "increased risk of bleeding due to trauma". Randomized to iv activated Protein C 24 μg/kg/h vs. placebo × 96 hours.	Increased circulating protein C levels. No impact on PAI-1.	No effect on plasma IL-6.	No benefit in ventilator-free days, 60-day mortality, or any other measured outcome.
Ranieri et al. [76]	1697 adult patients with septic shock. Excluded TBI or trauma with increased risk of severe bleeding. Randomized to iv activated protein C 24 μg/kg/h vs. placebo × 96 hours.	Increased circulating protein C activity.	Not reported.	No benefit in 28, 90 day mortality. No change in sequential organ failure assessment (SOFA) scores.
Heparin				
Bandeshe et al. [79]	214 adult patients (3 ICUs) mechanically ventilated >48 hours (9% trauma). 94% of subjects enrolled at one site. Randomized to nebulized heparin (5000 units every 6 hours), nebulized saline, or usual care.	No effect on systemic coagulation. Airspace coagulation not reported.	Airspace inflammation not reported.	No effect on ventilator-induced pneumonia or organ-specific SOFA. Nebulized saline group had higher risk of ARDS development than heparin or usual care groups.

(continued)

Table 38.1 (continued)

Study	Study population/intervention	Impact on coagulation	Impact on inflammation	Impact on clinical outcomes
Thrombomodulin				
Vincent et al. [71]	741 adult patients (33 ICUs across 17 countries) with sepsis and suspected DIC. Excluded if recent history of significant bleeding. Randomized to iv thrombomodulin (0.06 mg/kg/d) × 6 days or placebo.	Lower plasma D-dimer, prothrombin fragment 1.2, thrombin: antithrombin complexes.	No difference in systemic inflammatory markers.	No effect on 28 day mortality or indices of organ failure (including ventilator-free days).
Vincent et al. [72]	816 adult patients (159 sites across 26 countries) with sepsis, cardiovascular and/or respiratory failure, and both INR >1.4 and platelets 30–150 × 10^9/L. Excluded patients with TBI or recent (3 month history) of trauma associated with increased risk of bleeding. Randomized to iv thrombomodulin (0.06 mg/kg/d) × 6 days or placebo.	Lower plasma D-dimer, prothrombin fragment 1.2, thrombin: antithrombin complexes.	Not reported.	No effect on 28 day mortality. Indices of organ failure not reported.

[30, 68, 69], exogenous administration of anticoagulants have been largely disappointing in trials of sepsis or ARDS (Table 38.1). Intravenous administration of antithrombin III [70] or thrombomodulin [71, 72] failed to improve outcomes in sepsis despite successfully inducing systemic anticoagulation. While the 2001 PROWESS study demonstrated a substantial mortality benefit of intravenous activated protein C in sepsis [73], this benefit did not translate to ARDS patients [74] despite the ability of activated protein C to successfully attenuate pulmonary coagulopathy [74, 75]. Ultimately, a follow-up study of activated protein C in septic shock failed to reproduce the promising findings of PROWESS [76], leading to removal of this drug (drotrecogin alpha) from the market. Despite some success of systemic heparin in preclinical models [68], human studies of intravenous heparin in sepsis and lung injury are sparse and largely inconclusive [77–79].

Given concerns for the bleeding risk of systemic anticoagulation (in trauma and nontrauma critical illness), several studies have sought to determine the feasibility and efficacy of inhaled anticoagulants in lung injury. Despite promising preclinical studies, human trials failed to demonstrate meaningful benefit for patients with ARDS or at-risk for ARDS [79–81].

- *Administration of exogenous fibrinolytics***.** Given concerns about the hemorrhagic risk of systemic fibrinolysis in critically ill patients, there has been interest in the use of nebulized fibrinolytics in overcoming the upregulation of PAI-1 during lung injury. However, studies of inhaled fibrinolytics in animal models of ARDS yielded mixed results. While inhaled tissue plasminogen activator attenuated IL-1-induced ARDS [82], nebulized fibrinolytics only induced transient (and modest) benefit after cotton-smoke-induced lung injury [83]. The efficacy of nebulized fibrinolytics was notably modified by ventilator strategy and frequency of suctioning [83]. Despite these conflicting preclinical data, a small clinical study of patients with refractory ARDS (using a nonrandomized control group with a substantial 90% mortality) suggested substantial benefit of nebulized fibrinolytics as a rescue therapy [84].

The use of systemic fibrinolytics is relatively contradicted in patients with multi-

trauma, reflecting a heightened risk of hemorrhage. However, limited preclinical and clinical data suggest that intravenous fibrinolytics might be administered safely to patients with trauma-associated ARDS, with potential for efficacy. Hardaway and colleagues demonstrated that intravenous fibrinolytics, administered to anesthetized pigs 4 hours after (nonhemorrhagic) blunt soft tissue trauma, prevented the onset of acute lung injury [85]. The investigators followed up on these intriguing preclinical data with a phase I (observational) study of 20 severe ARDS patients (ages 3–50) admitted to two ICUs in the United States and Germany [86]. Twelve of the enrolled patients were posttrauma, 5 of whom had experienced antecedent hemorrhage. In these patients, 24-hour infusion of urokinase coincided with improved arterial oxygenation without causing bleeding; this infusion could be safely repeated if recurrent hypoxia occurred. These data, while intriguing, require careful validation in larger studies of patients at low risk of bleeding. Alternative approaches to promote fibrinolysis, such as manipulations of annexin A2 signaling [87], may be effective with an improved safety profile.

Lessons Learned From Failed Clinical Trials of Anticoagulants in Critical Illness

It is important to acknowledge that the failure of numerous trials of coagulation-targeted therapeutics could support the "null hypothesis" that coagulation is *not* a meaningful contributor to human ARDS. However, given the presence of substantial mechanistic and observational data supporting a role for pulmonary coagulation in the pathogenesis of lung injury, failures of these randomized trials may alternatively reflect scientific and practical challenges that commonly complicate trials of ARDS pharmacotherapies:

- *In vivo drug efficacy*. It is possible that administered anticoagulants/profibrinolytics do not effectively penetrate the chaotic multicellular milieu of an injured alveolus. This concern is partially offset by evidence that interventions which failed to improve patient outcomes nevertheless successfully impacted systemic indices of host coagulation (Table 38.1). However, agents capable of inducing systemic anticoagulation may still not adequately interrupt fibrin formation within the injured lung. Hypoxic vasoconstriction (augmented by pulmonary microthrombi) may shunt intravenously delivered agents away from injured alveoli, while inhaled drugs may preferentially deposit in normal, compliant airspaces.
- *Compartmentalization of signaling pathways*. As previously described in the study of tissue factor in ARDS, the location at which extrinsic coagulation occurs may impact its biologic impact. Blockade of local, alveolar epithelial production of tissue factor tends to worsen outcomes in preclinical models, while systemic blockade of intravascular coagulation tended to improve outcomes. However, these disparate, compartmental-specific effects of coagulation are not clearly recapitulated in therapeutic studies of anticoagulants, which consistently failed to improve outcomes (Table 38.1) even when administered intravascularly to patients with systemic (intravascular) illnesses such as sepsis.
- *Complexity of the extrinsic coagulation cascade*. Components of the extrinsic coagulation cascade are mechanistically promiscuous, interfacing with a variety of homeostatic and pathogenic pathways implicated in lung injury and repair. Thrombomodulin, simplistically an activator of protein C, also inhibits thrombin-induced cleavage of PAR receptors, interrupts damage-associated molecular patterns, directly shapes mitogen-activated protein (MAP) kinase and nuclear factor-kappa B (NF-κB) signaling, and can dramatically induce TAFI production [88, 89].

Heparin, an activator of antithrombin III, also binds avidly to circulating protein ligands, promoting both injurious and reparative growth factor pathways [63]. Fibrinolytics, in addition to promoting fibrin breakdown, can influence epithelial apoptosis, epithelial-

mesenchymal transition, and coagulation activation [20]. Given these highly interdependent yet widely disparate effects of components of the extrinsic coagulation cascade, it seems unlikely that single coagulation-targeted agent would consistently impart beneficial effects across the chaotic signaling milieu of an injured alveolus. Given these challenges, there is increasing interest in using multiple agents to simultaneously target both coagulation and inflammation, akin to the synergistic benefit of simultaneous DNAse and tPA in managing empyema [90].

- *Assumption that sepsis pathogenesis is relevant to trauma-associated ARDS*. While this review has endeavored to focus on data derived from studies of trauma-associated ARDS, this literature remains sparse. As such, much of the current understanding of injurious pulmonary coagulation continues to be derived from studies of infection-induced lung injury (e.g., sepsis or pneumonia), reflecting the high prevalence of infection as a cause of human ARDS [2]. Furthermore, randomized controlled trials of anticoagulant therapies often exclude trauma patients under the guise of avoiding hemorrhage (Table 38.1). As trauma-induced ARDS likely reflects a distinct pathogenic process than infection-induced ARDS, the failures of multicenter studies of anticoagulants in ARDS may simply not apply to trauma-associated ARDS.
- *ARDS endotypes*. The failure to impact ARDS outcomes is not unique to coagulation-targeted therapies. Indeed, there exists no known clinically efficacious, pathogenesis-targeted therapy in ARDS. These failures may represent clinical trials' tendency to falsely homogenize ARDS as a single disease state. Just as a single chemotherapeutic would be unlikely to treat all forms of cancer, administration of a single drug to all types of ARDS would be unlikely to consistently produce beneficial outcomes. Calfee and colleagues, using baseline patient data collected from two ARDS Network studies (of which 13% and 9% of patients were trauma-associated ARDS, respectively), performed unbiased latent class analysis to determine that clinically diagnosed ARDS in fact represents two distinct disease states [91]. One phenotype, milder in severity and accounting for two thirds of ARDS patients, was characterized by elevated circulating PAI-1. The other, more severe phenotype was characterized by suppressed circulating protein C. These findings have since been validated in other ARDS Network studies [92, 93] and by other groups [94]. It is unknown if these subphenotypes would similarly exist in a trauma-enriched ARDS cohort, or if the distinct coagulation signature of each phenotype would lead to divergent responses to coagulation-focused therapies.

Summary

Investigating the role of coagulation in the onset and progression of trauma-associated ARDS reveals challenges common to many studies of ARDS pathogenesis. While activation of intravascular and intraalveolar coagulation has been repeatedly observed within the lungs of patients and animals after trauma, the signaling pathways responsible for these changes are highly complex, incorporating multifaceted interactions with processes of lung inflammation and repair. Accordingly, most studies of coagulation-targeted therapies have failed to convincingly impact patient outcomes. There is reason for optimism, however—an emerging appreciation for the etiologic (traumatic vs. nontraumatic) and phenotypic (PAI-1-high vs. protein C-low) heterogeneity of ARDS suggests that future studies may be able to select patients most likely to respond to anticoagulant or profibrinolytic therapies. Such precision medicine approaches may substantially improve the care of patients with posttraumatic ARDS.

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Management of Chronically Anticoagulated Patients

39

Jerrold H. Levy

Introduction

Critically ill patients who present following trauma or are in an intensive care unit (ICU) often receive or require anticoagulation for multiple reasons that include stroke prevention, atrial fibrillation (AF), mechanical valves, and venous thromboembolic disease [1, 2]. The need for therapy and type of anticoagulant vary depending on whether patients have arterial or venous thromboembolic issues. Both these issues are important following traumatic injury and have significant implications for patient management. There are also important interactions and links between coagulation and other physiologic responses, including inflammation, that are beyond the scope of this chapter.

Many types of oral anticoagulant therapies are available that include (vitamin K antagonists [VKAs, e.g., warfarin] and the direct nonvitamin K oral agents (apixaban, dabigatran, edoxaban, rivaroxaban), and platelet inhibitors (thienopyridines [e.g., clopidogrel, prasugrel] and nucleoside analogs [e.g., ticagrelor]) [3–5]. Occasionally, patients may also be receiving low-molecular-weight heparin (LMWH) as self-administration for chronic therapy. Patients may also be receiving an anticoagulation agent for thromboprophylaxis and have concomitant coronary artery disease and are chronically treated with antiplatelet therapy.

All of the available anticoagulant therapies prevent thrombus formation in pathologic states but can also exacerbate bleeding following traumatic or surgical injury. Patients tend to have significant morbidity and mortality owing to their procoagulant diseases, but the anticoagulant therapy they receive creates an important acquired hemostatic defect due to alterations in the physiologic procoagulant/anticoagulant equilibrium [1, 4]. Trauma alone produces a complex coagulopathic state, which is the subject of this book. The acquired hemostatic defect in the anticoagulated patient may further contribute to the trauma-induced coagulopathy that occurs following trauma or during emergency surgery.

Critical to understanding the effects of chronic anticoagulation is that hemostasis is far more complex than the simplified coagulation cascades that most clinicians have learned in medical school [6–10]. The complex equilibrium among blood cells, platelets, coagulation factors, natural inhibitors of coagulation, and the fibrinolytic system is altered by a procoagulant state, and anticoagulant therapy is instituted to alter the pathologic responses of hypercoagulability [11]. Patients may also have arterial disease and may have

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stents in their coronary or cerebral circulation, adding to the complexity of the situation. Arterial thrombi are mediated by platelet responses although coagulation, hemostasis, and thrombus formation involve important cross talk between endothelial cells, platelets, and coagulation proteins [8, 12].

Arterial injury serves as a procoagulant focus for clot formation due to platelet adhesion, activation, and aggregation. This is a normal physiologic process but can be pathologic in the case of plaque rupture, myocardial infarction, and/or stroke [13, 14]. Platelets normally circulate in an inactivated state, but following activation they express glycoprotein IIb/IIIa (GpIIb/IIIa) receptors, allowing fibrinogen to bind, cross-link, and aggregate platelets to form a thrombus [14, 15]. Vascular injury causes thrombin formation but also platelet activation, and the platelet–fibrinogen plug is formed. Since platelets have a pivotal role in the pathogenesis of thrombosis after plaque rupture, antiplatelet agents including aspirin, thienopyridines (clopidogrel, prasugrel), nucleoside analogs (ticagrelor), and the GpIIb/IIIa inhibitors reduce adverse events that are associated with plaque rupture [16, 17].

Patients may present with underlying hemostatic disorders following trauma, during surgery, or in the ICU because of chronic anticoagulation and/or antiplatelet therapy [18]. All therapies that prevent clot formation in pathologic states also interfere with normal hemostasis, an important mechanism that protects patients from excessive bleeding [19, 20]. Therefore, in cases of trauma, or prior to emergency surgery, these patients require rapid anticoagulation reversal. It is worth noting that a patient's anticoagulant therapy will need to be reinitiated posttrauma and surgery; prophylaxis for deep vein thrombosis (DVT), pulmonary embolism (PE), and venous thromboembolism (VTE) may also be required once hemostasis is established and hemorrhage is controlled.

This chapter focuses on chronic oral anticoagulant therapies and the therapeutic approaches used to treat or prevent bleeding in patients in cases of trauma or emergency surgery.

Anticoagulation

Anticoagulation is based on inhibiting both thrombin and platelet activation [4, 10, 21–23]. Thrombin is a potent procoagulant that generates fibrin from soluble fibrinogen, activating coagulation factors (F) V and VIII, and platelets [8]. Activated platelets adhere to injured vascular endothelia, express GpIIb/IIIa receptors, aggregate, and further increase generation of thrombin [24]. Due to the complex humoral and cellular amplification systems linking both hemostatic and inflammatory responses, there are multiple pathways to produce thrombin and prothrombotic effects [10, 19]. Anticoagulants currently used orally to prevent clot formation are considered in the following sections.

Oral Anticoagulants

VKAs: Warfarin

Warfarin is the only oral VKA available in the USA. It is an effective anticoagulation agent that acts by inhibiting vitamin K epoxide reductase, the enzyme that posttranslationally modifies the vitamin K–dependent coagulation proteins (FII [prothrombin], FVII, FIX, and FX), converting them to their active forms. Warfarin's onset of action is slow, and it takes several days to decrease coagulation factors to the ~20–45% level that is required for a therapeutic international normalized ratio (INR) of 2–3 [25]. Despite the increased availability of newer agents, warfarin and other VKAs continue to be widely used due to the extensive experience that has been accrued with these agents in a broad spectrum of indications, including mechanical cardiac valves and ventricular assist devices.

Nonvitamin K Oral Agents: Apixaban, Dabigatran, Edoxaban, Rivaroxaban

The new target-specific nonvitamin K oral anticoagulation agents have a rapid onset of action, and therapeutic anticoagulation is achieved

within hours of administration. Dabigatran is a direct thrombin (FIIa) inhibitor, while apixaban, edoxaban, and rivaroxaban are direct FXa inhibitors; in contrast to LMWH, FXa inhibitors exert their anticoagulant effect independently of antithrombin [2, 5]. The nonvitamin K oral anticoagulants (NOACs) require dose adjustments in patients with renal failure. These agents will be considered separately in the following sections.

Dabigatran Etexilate (Pradaxa®, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT, USA)

Dabigatran etexilate is an oral, direct thrombin inhibitor currently approved in the USA to reduce the risk of stroke and systemic embolism in patients with nonvalvular AF; for the treatment of DVT and PE in patients who have been treated with a parenteral anticoagulant for 5–10 days; to reduce the risk of recurrence of DVT and PE in patients who have been previously treated; and for the prophylaxis of DVT and PE in patients who have undergone hip replacement surgery [26]. Dosing should be adjusted for patients with renal dysfunction [26]. The capsule is specially formulated and cannot be altered or crushed for administration in an ICU setting.

Apixaban (Eliquis®, Bristol-Myers Squibb Company, Princeton, NJ, USA)

Apixaban is an FXa inhibitor anticoagulant approved in the USA to reduce the risk of stroke and systemic embolism in patients with nonvalvular AF; for the prophylaxis of DVT, which may lead to PE, in patients who have undergone hip or knee replacement surgery; and for the treatment of DVT and PE, and the reduction in the risk of recurrent DVT and PE following initial therapy [27]. The apixaban formulation can be crushed and given through a feeding tube if needed in the ICU setting.

Rivaroxaban (Xarelto®, Janssen Pharmaceuticals Inc., Titusville, NJ, USA)

Rivaroxaban is a direct-acting oral, FXa inhibitor that, unlike heparins, does not require antithrombin [28]. Rivaroxaban has the broadest indications of all of the NOACs and is approved in the USA for reducing the risk of stroke and systemic embolism in patients with nonvalvular AF; for the treatment of DVT and PE, and for the reduction in the risk of recurrent DVT and/or PE; for the prophylaxis of DVT, which may lead to PE in patients undergoing knee or hip replacement surgery; and in combination with aspirin, to reduce the risk of major cardiovascular events (cardiovascular [CV] death, myocardial infarction [MI] and stroke) in patients with chronic coronary artery disease (CAD) or peripheral artery disease (PAD).

Measurement of NOAC-Induced Anticoagulation

One of the major advantages of the NOACs is that coagulation monitoring is not necessary for their management. In addition, overall dosing recommendations are simple, based on renal function and indication, i.e., VTE prophylaxis, thromboembolism treatment, or prevention of stroke in patients with AF. Nevertheless, specific quantitative testing to determine the anticoagulant effect of NOACs may be required following trauma, prior to emergency surgery, and/or in cases of significant bleeding. When managing a patient in one of these scenarios, it is helpful to determine when the last dose of anticoagulant therapy was given, especially in cases of patients needing urgent surgery or procedural interventions; this is also useful in assisting with the interpretation of coagulation test results. However, obtaining an accurate medical history can sometimes be difficult for a trauma patient or in the emergency department (ED). Knowing the renal function at the time of coagulation testing is also essential since renal excretion is a major component of the elimination mechanism for

Table 39.1 Pharmacokinetics and monitoring of NOACs [1, 28–31]

	Dabigatran	Apixaban	Edoxaban	Rivaroxaban
Plasma peak level	2 h after ingestion	1–4 h after ingestion	1–2 h after ingestion	2–4 h after ingestion
Plasma trough level	12–24 h after ingestion	12–24 h after ingestion	12–24 h after ingestion	16–24 h after ingestion
PT	Not for routine monitoring	Cannot be used	May be prolonged	May be prolonged: may indicate excess bleeding risk
INR	Cannot be used	Cannot be used	Cannot be used	Cannot be used
aPTT	At trough >2× ULN suggests excess bleeding risk	Cannot be used	Cannot be used	Cannot be used
dTT	At trough >200 ng/ml or >65 s: excess bleeding risk	Cannot be used	Cannot be used	Cannot be used
Anti-FXa chromogenic assays	Not applicable	Quantitative if calibrated: no data on threshold values for bleeding or thrombosis	Quantitative if calibrated: no data on threshold values for bleeding or thrombosis	Quantitative if calibrated: no data on threshold values for bleeding or thrombosis
ECT	At trough: ≥3× ULN: excess bleeding risk	Not affected	Not affected	Not affected

Data from Refs. [1, 2, 4, 26–29]
aPTT activated partial thromboplastin time, *dTT* diluted thrombin time, *ECT* ecarin clotting time, *F* factor, *INR* international normalized ratio, *N/A* not applicable, *PT* prothrombin time, *ULN* upper limit of normal

some of these agents, particularly dabigatran (Table 39.1) [29–31].

Overall, as screening and qualitative assessments of effect but not definitive tests, the activated partial thromboplastin time (aPTT) is prolonged by dabigatran. The prothrombin time (PT) is minimally affected with apixaban, rivaroxaban, and other FXa inhibitors and is not reliable in determining drug effects unless a specifically calibrated assay is used [31–33]. Following trauma, multiple factors influence the PT and it is frequently prolonged even in the absence of FXa inhibitors. Specific quantitative tests for FXa inhibitors exist but may not be readily available in the urgent situation of trauma or in the ED. Recommendations suggest that point-of-care testing should not be used to assess INR in patients receiving NOACs although drug specific testing is under development [34].

The coagulation tests used to manage NOACs in trauma or emergency situations are summarized in Table 39.1 and are discussed further in the following sections.

Measuring and Monitoring Dabigatran

The anticoagulant effects of dabigatran can be monitored using thrombin clotting time/thrombin time (TT) and aPTT values, although TT is generally preferred [29–34]. The aPTT provides an evaluation of dabigatran effect, but a normal aPTT does not exclude its presence. The INR and PT are not affected by dabigatran at therapeutic concentrations and should not be used for assessing its anticoagulant effect. However, in cases where dabigatran levels are several times higher than therapeutic levels, the PT/INR can be elevated. The ecarin clotting time can be used to assay direct thrombin inhibitors, but this is not used in most centers, especially in the USA. Most centers can measure TT, and a normal TT excludes a dabigatran effect. A specialized assay that uses a diluted TT is helpful for specific measurement of dabigatran levels when needed. This assay is available in some centers as the

Hemoclot® test and can exclude a clinically relevant anticoagulant effect.

Measuring and Monitoring Apixaban, Edoxaban, and Rivaroxaban (FXa Inhibitors)

Therapeutic levels of FXa inhibitors have minimal effects on the PT, and as noted, multiple factors can also prolong the PT/INR in trauma patients. The effects of FXa inhibitors on PT also depend on the type of assay, the particular agent, and the PT reagents used. All clotting tests use a specific tissue thromboplastin. INR is used specifically for warfarin and other VKAs and cannot be used for determining the effects of apixaban, rivaroxaban, or other FXa inhibitors. The aPTT is also not appropriate for determining the effects of the FXa inhibitors. The most sensitive assay to monitor FXa inhibitors is a drug specific anti-Xa chromogenic assay. This is similar to the anti-FXa assay used to monitor LMWH therapy but is calibrated to the specific FXa inhibitors [29–34].

Emergency Management of Patients on Oral Anticoagulants

Following trauma or prior to emergency surgery, urgent anticoagulation reversal or management plans are needed, especially in patients with acute bleeding. Specific reversal therapies for the NOACs are now currently available and will be considered and are part of a multimodal strategy for managing these emergencies. Managing bleeding and therapeutic approaches in this patient population are discussed in the following sections.

Managing Bleeding Associated with Warfarin and Other VKAs

Various treatment options for VKA reversal are available depending upon the urgency for reversal [25]. Vitamin K supplementation is important in the trauma and/or bleeding patient; however, it will not immediately reverse the anticoagulant effect of VKAs. Therefore, additional therapies are needed in emergency situations, as detailed in the American College of Cardiology Task Force on Expert Consensus Decision Pathways [36]. Fresh frozen plasma (FFP) is often used for VKA reversal. However, transfusion of FFP is unable to restore the INR to baseline, and usually only reduces INR to ~1.4–1.5 [25]. Furthermore, transfusion of FFP is associated with various safety issues, such as volume overload. Prothrombin complex concentrates (PCCs) can also be used for urgent VKA reversal. In the USA, PCCs are available as 4-factor (4F-PCC; Kcentra®, CSL Behring, King of Prussia, PA), or 3-factor (3F-PCC; Profilnine® SD, Grifols Biologicals Inc. Los Angeles, CA, USA and Bebulin®, Baxter AG, Vienna, Austria). The 3F-PCCs do not contain clinically relevant amounts of FVII.

The management of warfarin-associated bleeding in trauma or emergency surgery settings has been greatly facilitated by the advent of balanced PCCs that contain all four vitamin K-dependent factors [25]. Multiple guidelines recommend the use of 4F-PCCs for urgent warfarin reversal [25]; however, clinicians still continue to use fresh frozen plasma/plasma for reversal in the USA. Therefore, warfarin reversal remains a clinical challenge.

Sarode et al. conducted a prospective clinical trial comparing a nonactivated 4F-PCC (Kcentra®, containing coagulation factors II, VII, IX, and X and proteins C and S) with plasma for urgent VKA reversal in patients with acute major bleeding [36]. Their primary analyses examined whether 4F-PCC was noninferior to plasma for two coprimary endpoints of hemostatic efficacy (assessed over 24 h from start of infusion) and rapid INR correction (INR $\leq$ 1.3 at 0.5 h after end of infusion). The intention-to-treat efficacy population consisted of 202 patients (4F-PCC: n = 98; plasma: n = 104). Baseline characteristics, including baseline INR values, were similar between groups. 4F-PCC was noninferior to plasma for hemostatic efficacy and both noninferior and superior to plasma for rapid INR

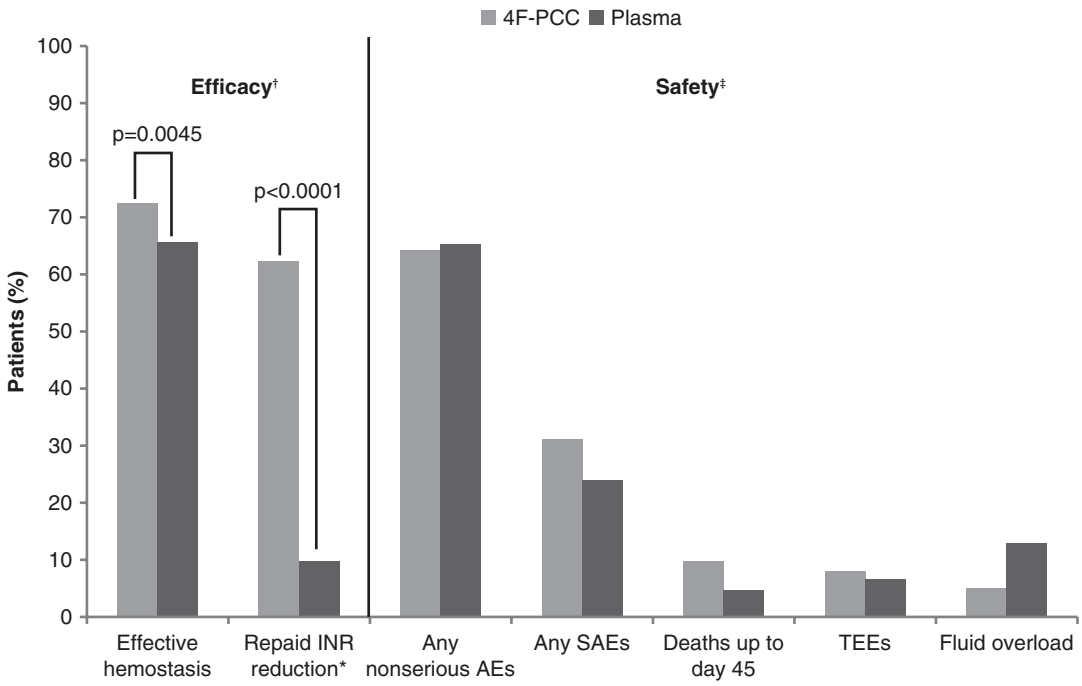


Fig. 39.1 Urgent warfarin reversal in patients with acute major bleeding: results from a randomized controlled trial. 4F-PCC four-factor prothrombin complex concentrate, AE adverse event, INR international normalized ratio, SAE serious adverse event, TEEs thromboembolic events. *Rapid INR reduction: INR ≤ 1.3 at 0.5 h postinfusion start; †intent-to-treat efficacy population (4F-PCC: n = 98; plasma: n = 104); —reported p-values are Farrington–Manning p-values for noninferiority; ‡intent-to-treat safety population (4F-PCC: n = 103; plasma: n = 109). (Figure adapted from Sarode et al. [36])

reduction. Effective hemostasis was achieved in 72.4% and 65.4% of patients in the 4F-PCC and plasma groups, respectively (Fig. 39.1). Rapid INR reduction was achieved in 62.2% and 9.6% of patients in the 4F-PCC and plasma groups, respectively (Fig. 39.1). Mean plasma levels of vitamin K-dependent factors were higher in the 4F-PCC group than in the plasma group from 0.5 to 3 h after the start of infusion ($p < 0.02$). There was no significant difference in the occurrence of adverse events, serious adverse events, thromboembolic events, and deaths between treatment groups, although fluid overload events occurred more frequently in plasma-treated than in 4F-PCC-treated patients (12.8% and 4.9%, respectively) (Fig. 39.1). The authors concluded that 4F-PCC is an effective alternative to plasma for urgent VKA reversal in patients with major bleeding events [36].

Managing Bleeding Associated with NOACs

As previously discussed, NOACs are administered without routine monitoring; however, specialized tests can be implemented to evaluate their anticoagulant effects in bleeding patients. To reiterate, for dabigatran, TT and diluted TT (Hemoclot® assays) are the most sensitive, while aPTT is a qualitative assessment of anticoagulation. The anticoagulant effects of FXa inhibitors are more difficult to measure and specific drug calibrated anti-FXa assays, such as those used for LMWH, are required. However, a LMWH assay can be used as a qualitative assay to determine the presence but not the level of a factor Xa inhibitor.

With NOACs, as with any anticoagulant agent, bleeding complications are a concern and antico-

agulation reversal may be required. Nevertheless, most studies suggest that patients fare better on NOACs compared with warfarin [37]. In one study of 27,419 patients treated with either dabigatran or warfarin for up to 3 years, 1034 patients had 1121 major bleeds. The 30-day mortality after the first major bleed was 9.1% in the dabigatran group compared with 13.0% in the warfarin group, and dabigatran-treated patients required a shorter ICU stay compared with patients who received warfarin [37]. Studies have also shown that bleeding profiles with NOACs, in particular those of intracranial and other life-threatening bleeding, are more favorable than those with warfarin [38, 39].

Specific antidotes to antagonize the anticoagulant effect of NOACs are currently available for dabigatran, a Fab fragment idarucizumab (Praxbind), and currently available for FXa inhibitors, andexanet alfa [1]. Dabigatran reversal with idarucizumab has been studied both in medical bleeding patients but also surgical, procedural, and trauma patients as well. Andexanet alfa was effective in the study of 352 patients receiving a factor Xa inhibitor but included only intracranial hemorrhage [64%] or gastrointestinal bleeding [26%] and was not tested in surgical patients [40]. The cost of andexanet is approximately $25,000/gram. The dosing depends on what FXa inhibitor the patient is receiving and when the last dose was administered, and factor Xa inhibitor levels for ~2–3 h, then levels return back to baseline as demonstrated in the clinical studies [1].

However, NOACs have a relatively short duration of effect in patients with normal renal function; therefore, discontinuation of NOACs can be sufficient in cases of mild bleeding or where surgery can be delayed. Nevertheless, in cases of major bleeding or where emergency surgery is required, reversal strategies should be implemented. Patients receiving NOACs and presenting with bleeding should be hemodynamically and hemostatically resuscitated, and therapy should be multimodal, as outlined in Fig. 39.2.

Multiple publications including guidance and guideline documents are now available to facilitate bleeding management in NOAC-treated patients [1, 25, 30, 34, 35]. Increasing case series and guidelines suggest that the off-label administration of PCCs to treat bleeding primarily in patients receiving FXa inhibitors [25, 41–44]. As a reminder, it should be noted that restoring hemostatic function may not necessarily equate to good clinical outcomes. Recommendations on NOAC-associated bleeding management are often based on guidance/guideline documents that include expert opinions; however, increasing information and the available of antidotes are greatly improving our management strategies rather than clinical experience. These recommendations will be considered in the following sections.

Managing Moderate-to-Severe Bleeding in NOAC-Treated Patients

Standard bleeding management strategies, such as hemodynamic/hemostatic support and mechanical/surgical hemostasis, can be implemented to treat NOAC-associated bleeding. Moreover, due to the relatively short half-lives of the NOACs compared with VKAs, coagulation is usually restored within 24–48 h of the last dose in patients with normal renal function. However, renal function may be acutely impaired in patients with shock. Special precautions should also be taken with elderly patients, especially those with intracoronary stents and with patients concomitantly receiving NOACs and antiplatelet drugs. Early utilization of a massive transfusion protocol restoring intravascular blood volume should be considered in these patients.

Dabigatran can be reduced using dialysis; however with the availability of idarucizumab, this technique is seldom used and not feasible in shock. Dialysis is not considered to be effective in patients receiving FXa inhibitors due to the high plasma binding of most FXa inhibitors (Table 39.1). The advent of idarucizumab, the specific reversal agent for dabigatran, minimizes the need for dialysis.

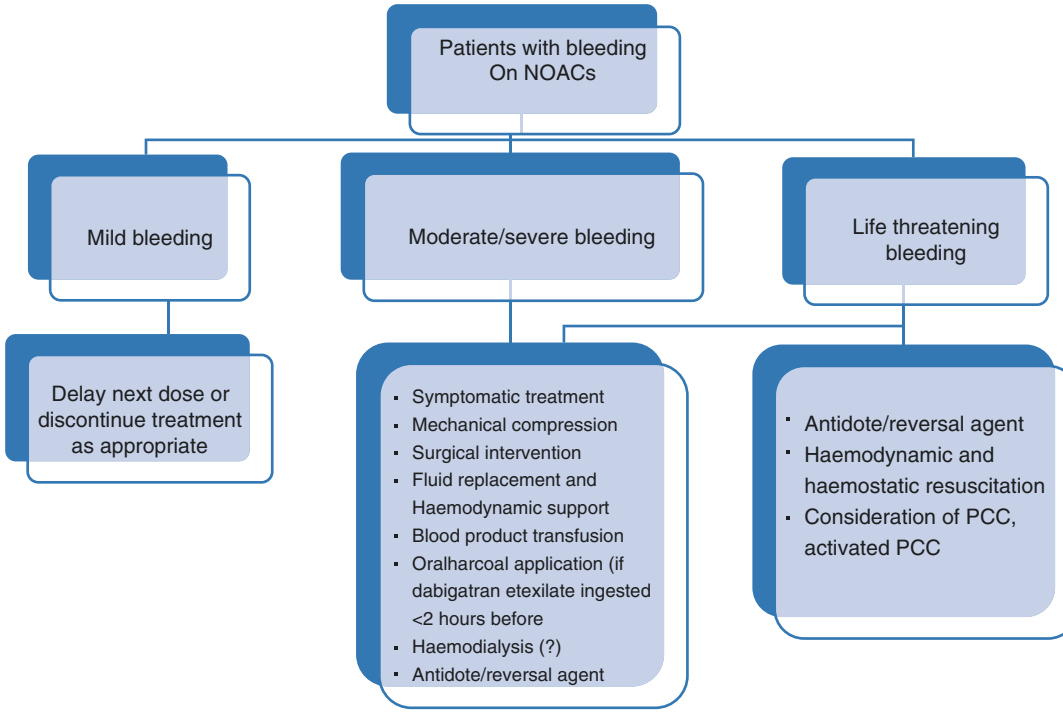


Fig. 39.2 Bleeding management strategies for NOAC-treated patients. In cases of mild bleeding, stopping or delaying the next dose should be considered. NOACs have relatively short half-lives, so when stopping the drug in patients with normal renal function, the anticoagulant effect rapidly decreases compared with warfarin. In patients with moderate-to-severe bleeding, standard therapeutic approaches should be considered, including supportive care that includes volume resuscitation, hemodynamic support with vasoactive therapy, blood product transfusions as determined by testing, and identification of bleeding source that may require surgical or another intervention. If the agents were taken within ~2 h of admission, administration of oral activated charcoal should be considered. For dabigatran, a specific monoclonal Fab fragment reversal agent is available (idarucizumab). Although hemodialysis can remove ~60% of the drug after several hours of dialysis, the use of idarucizumab simplifies the management strategy. Factor Xa inhibitors including apixaban, edoxaban, and rivaroxaban are highly protein bound and will not be cleared by hemodialysis. For patients with life-threatening bleeding, hemodynamic and hemostatic resuscitation should be considered, with therapy similar to a trauma patient including the use of a massive transfusion protocol. Based on current data as discussed in the manuscript, the use of either 3-factor or 4-factor prothrombin complex concentrates (PCCs), depending on their availability, should be considered as suggested in guidelines and other documents as reviewed in this chapter. The use of recombinant activated factor VIIa (rFVIIa) is not considered to be effective

Managing Life-Threatening Hemorrhage in NOAC-Treated Patients

In cases of major bleeding, it is critical that clinicians employ a massive transfusion protocol in addition to hemostatic management. Although this is second nature to trauma physicians, it may not be the case with others. A specific reversal agent for dabigatran, idarucizumab, a humanized monoclonal Fab fragment that selectively binds dabigatran and immediately reverses dabigatran-induced anticoagulation, has been studied in both surgery and trauma as noted in the REVERSE-AD study. Andexanet alfa is now approved for when reversal of anticoagulation is needed due to life-threatening or uncontrolled bleeding. Andexanet alfa is a bioengineered human factor Xa decoy protein that has been modified to delete the native catalytic activity, while retaining the high-affinity

binding of factor Xa inhibitors within the enzymatic active site. By binding to circulating factor Xa inhibitors, andexanet alfa makes endogenous factor Xa available to contribute to the coagulation cascade.

Current guidelines include PCCs as part of a multimodal approach in patients requiring urgent surgery or experiencing life-threatening bleeding.[1] Several large patient registries and observational studies including the Dresden NOAC registry, demonstrated the rates, management, and outcome of rivaroxaban-related bleeding. Of 1776 patients, 66 patients experienced a major bleeding event, and 6 patients received PCCs at doses that range from 18–47 units per kilogram. Only one patient had a significant improvement in coagulation parameters; five of the six patients demonstrated hemorrhage control. In a retrospective review of patients bleeding due to dabigatran or rivaroxaban, a median dose of PCC 40 IU/kg was administered in 3 out of 25 patients. All three patients had rivaroxaban-associated bleeds (one major, two life threatening) and administration of PCC successfully resolved the bleeding in all cases. With regards to the perioperative setting, a retrospective, multicenter study investigated patients who received 4F-PCC for treatment of the anticoagulation effects of FXa inhibitors when developing a pericardial effusion during or after atrial fibrillation ablation. In total, 11 patients were administered 4F-PCC. Two patients required further surgery for treatment of the pericardial effusion, while the other nine patients were hemodynamically stable and there was no recurrence of the pericardial effusion, demonstrating that 4F-PCC is an effective management option in this patient population [44].

The use of 4F-PCCs was recently reported in a prospective evaluation of 84 patients receiving rivaroxaban or apixaban who were treated with PCCs for major bleeding and evaluated for thromboembolic events and all-cause mortality within 30 days. PCCs were administered at a median dose of 2000 IU dose (1500–2000 IU) for patients with intracranial hemorrhage (n = 59; 70.2%) or gastrointestinal bleeding (n = 13;15.5%). Treatment to stop bleeding was considered effective in 58 (69.1%) and ineffective in 26 (30.9%) of treated patients. The majority of the patients with ineffective hemostasis had intracranial hemorrhage (n = 16; 61.5%), and two patients developed an ischemic stroke 5 and 10 days after PCC administration. A total of 27 (32%) patients died within 30 days; however, there was no control group in the report [41].

An additional report from Canada evaluated major bleeding in 66 apixaban or rivaroxaban-treated patients treated with 2000 units of PCCs and evaluated thromboembolism or mortality 30 days later. Using a specific evaluation scale, the investigators reported cessation of bleeding was as good in 65%, moderate in 20%, and poor/none in 15% of patients and included patients with intracranial hemorrhage or gastrointestinal bleeding. Overall reversal was considered to be effective in 68% of patients and ineffective in 32%, and mortality was 14% in 30 days, with 8% risk of thromboembolic events [42].

In a systematic review of current PCC use experience and reports for NOAC reversal, Piran evaluated the safety and effectiveness of a 4-factor prothrombin complex concentrate (4F-PCC) as an alternative for managing direct FXa inhibitor-related major bleeding in a systematic literature search conducted up to September 2018. Ten case series with 340 patients who received PCC for direct FXa inhibitor-related major bleeding were included. The pooled proportion of patients with effective management of major bleeding was 0.69 (95% confidence interval [CI], 0.61–0.76) in two studies using the International Society on Thrombosis and Haemostasis (ISTH) criteria and 0.77 (95% CI, 0.63–0.92) in eight studies that did not use the ISTH criteria; all-cause mortality was 0.16 (95% CI, 0.07–0.26), and thromboembolism rate was 0.04 (95% CI, 0.01–0.08). On the basis of evidence with very low certainty from single-arm case series, it is difficult to determine whether 4F-PCC in addition to cessation of direct oral FXa inhibitor is more effective than cessation of direct oral FXa inhibitor alone in patients with direct FXa inhibitor-related major bleeding [43].

Table 39.2 Management of bleeding or emergency surgery in DOAC-treated patients

Mild bleeding		Moderate to severe bleeding		Life-threatening bleeding		Emergency surgery
Identify and manage bleeding site Hold anticoagulant if necessary Restart anticoagulant as soon as possible	+	Resuscitation: Hemodynamic and hemostatic resuscitation Obtain coagulation test results. Calculate creatinine clearance Control source of bleeding: Identify source of bleeding and treat if possible Reversal: Consider reversal if there is ongoing bleeding (see next column)	+	Reversal of anticoagulant: Dabigatran: Idarucizumab (5 g by intravenous bolus) Apixaban, edoxaban, or rivaroxaban Consider andexanet alfa if available 4-factor PCC (25–50 U/kg) If there is ongoing bleeding despite PCC, consider activated PCC (50 U/kg) With massive or uncontrollable hemorrhage, initiate massive transfusion protocol Coagulation tests including viscoelastic monitoring Consider tranexamic acid (1 g intravenously)	I I I I I I I I I I I I I I I	Measure drug levels if possible, but do not wait for test results if surgery is urgent Reverse dabigatran with idarucizumab Consider off-label use of andexanet administration for reversal of apixaban, edoxaban, or rivaroxaban either before surgery, and/or 4-factor PCC during or after surgery if there is excessive bleeding as part of a multimodal strategy

Modified from Levy et al. [1]

In instances of life-threatening bleeding, prohemostatic agents such as PCCs should also be considered, especially for bleeding related to the Xa inhibitors, or as part of a multimodal strategy (Table 39.2) [41–44]. The use of PCCs continues to be reported as part of a multimodal strategy both in surgical patients and following traumatic injury, and PCCs are recommended in current guidance documents for managing bleeding [45].

Multimodal Therapy

In bleeding patients, additional therapy should be considered in addition to a specific antidote when available that includes monitoring the coagulopathy, repletion of other coagulation factors, antifibrinolytics (e.g., tranexamic acid), and allogeneic transfusions should be considered as part of a multimodal therapeutic approach to manage NOAC-associated bleeding. Plasma/FFP may be indicated as part of a massive transfusion protocol along with other factors but not specifically for NOAC reversal. Neither vitamin K nor protamine has any role in managing NOAC-related bleeding. Although specific outcome data in actively bleeding patients receiving NOACs is still evolving, based on the information available to date and current reports, the administration of PCCs should be considered as part of multimodal therapy when major and life-threatening bleeding occurs. Based on the growing literature reported with PCCs in patients, ~2000 units (~ 25–35 units/kg) in adults appears to be an appropriate starting dose when an antidote/specific reversal agent is not available. The role of rFVIIa (NovoSeven®, Novo Nordisk A/S, Bagsværd, Denmark) does not seem clinically relevant based on current information. As previously noted, idarucizumab has been studied in surgical, procedural, and trauma patients while andexanet alfa has not been studied.

Platelet Inhibitors

Inhibiting platelet activation is critical for the management of patients with ischemic cardiovascular disease and/or atherosclerotic vascular disease [46–48]. As with oral anticoagulants, platelet inhibitors/antiplatelet agents pose increased risks for bleeding and should also be considered anticoagulants.

Antiplatelet agents differ in their modes of action, potency, onsets of action, and indications. Aspirin is an irreversible platelet cyclooxygenase and thromboxane A2 inhibitor but is also a relatively weak antiplatelet agent [49] and resistance can occur. More potent antiplatelet agents include the $P2Y_{12}$ receptor inhibitors and GpIIb/IIIa receptor antagonists (abciximab, tirofiban, eptifibatide).

The $P2Y_{12}$ receptor inhibitors include clopidogrel, prasugrel, and ticagrelor and have become the mainstays of antiplatelet therapy. These agents act to inhibit platelets by selectively and irreversibly binding to the $P2Y_{12}$ receptor, inhibiting the adenosine diphosphate (ADP)-dependent mechanism of GpIIb/IIIa receptor expression and platelet activation [46–48, 51, 52].

Clopidogrel is more commonly used than prasugrel or ticagrelor, but there is limited information available on how to monitor its effects and manage the patients receiving this therapy. Furthermore, resistance can occur because it is a prodrug and requires metabolism to be transformed to its active form. It is worth noting that clopidogrel decreases peak thrombin generation [53] and prolongs R-time on thromboelastography, suggesting a delay in thrombin generation [54]. Prasugrel [55] is one of the newer antiplatelet agents and has the advantage of increased potency and potentially a lower rate of resistance compared with clopidogrel [52]. It also has less pharmacologic variation in activation [56]. Prasugrel strongly inhibits ADP-induced platelet aggregation, P-selectin expression, and thrombin receptor agonist peptide (TRAP)-induced microparticle formation [56]. Ticagrelor is a direct-acting antiplatelet agent that should be considered similar to any P2Y12 inhibitor except it is an active drug and not a prodrug like clopidogrel and so the effect is consistent [57].

Dual-antiplatelet therapy with aspirin and clopidogrel is standard care following revascularization by percutaneous coronary intervention with stent insertion. This dual therapy is recommended for up to 4 weeks after intervention for bare-metal stents and for 6–12 months after intervention for drug-eluting stents [46–48]. Thus, patients may be receiving these drugs chronically following trauma.

In nontrauma patients, Vincenzi noted 45% complication rate and 5% mortality rate in patients undergoing noncardiac surgery after coronary artery stenting [55]. This high complication rate is likely due to discontinuing antiplatelet drugs, underlying cardiovascular disease, and multiple other factors that have a major influence on outcome. Patients presenting with coronary stents placed within 1 year and requiring surgery are at great risk for adverse events. In the study by Vicenzi et al. conducted in 103 patients who had received a coronary artery stent ≤1 year prior to surgery, 44.7% developed complications after surgery and 4.9% of the patients died. All except two (bleeding only) adverse events were of cardiac nature, and most of the complications occurred early after surgery. Patients with recent stents (<35 days before surgery) had 2.11-fold greater risk of experiencing an adverse event compared with those who had undergone percutaneous cardiac intervention more than 90 days before surgery [58]. The clopidogrel package insert suggests that if a patient is to undergo elective surgery and an antiplatelet effect is not desired, therapy should be discontinued 5 days before surgery. However, if patients bleed, treatment strategies or assays to monitor the effects of clopidogrel have not been established.

Managing Bleeding in Patients Receiving Antiplatelet Agents

Patients taking $P2Y_{12}$ receptor inhibitor/thienopyridine antiplatelet agents present following trauma or requiring emergency surgery with increasing frequency due to the extensive use of drug-eluting coronary stents. In cases of acute bleeding following trauma, urgent reversal of

antiplatelet agents may be required. Despite this concern, there are no approved or validated treatments for rapidly reversing anticoagulation in these patients. As a result, procoagulant therapies may be required in these situations. One commonly employed strategy is to transfuse allogeneic platelets. However, the efficacy and safety of this practice are unknown and there have been no randomized controlled trials conducted to date. One of the problems for managing platelet dysfunction is that most platelet function tests cannot be used in the trauma, bleeding, or postoperative patient due to dilutional thrombocytopenia and other coagulation abnormalities.

In the life-threatening bleeding patient, the off-label user VIIa may have potential for reversing $P2Y_{12}$ receptor inhibition. The concept behind this is based on the clinical use and US Food and Drug Administration (FDA) approval of rFVIIa (NovoSeven®) for treating bleeding episodes and perioperative management in patients with Glanzmann's thrombasthenia with refractoriness to platelet transfusions, with or without antibodies to platelets. Glanzmann's thrombasthenia is a rare genetic bleeding disorder characterized by defective or low levels of GpIIb/IIIa, a receptor for fibrinogen, resulting in platelet dysfunction. The defect in platelet function is similarly produced by clopidogrel, prasugrel, and ticagrelor [56].

rFVIIa has been shown to restore thrombin generation in clopidogrel-treated blood samples, shorten thrombin generation lag time in patients who had been treated with aspirin and clopidogrel [56], and in blood samples treated with clopidogrel or prasugrel's active metabolite [57, 58]. These findings are all consistent with the desired procoagulant effect of rFVIIa.

Although these in vitro data using rFVIIa are promising, caution must be exercised when extrapolating these data to clinical scenarios. It is possible that the increase in thrombin generation is purely an in vitro phenomenon. Also, the off-label use of rFVIIa has been the subject of persistent safety concerns, with two recent reviews describing an increased risk of arterial thrombosis and stroke in patients receiving off-label rFVIIa [59, 60]. Nevertheless, a slightly increased thrombotic risk may be acceptable in patients who have life-threatening bleeding and require salvage therapy. Thus, rFVIIa might be a useful salvage treatment in patients taking antiplatelet agents who present with life-threatening refractory bleeding. However, extreme caution should be taken when using rFVIIa to reverse the effect of antiplatelet drugs in less acute scenarios.

The Future

Although the NOACs have a lower risk of major bleeding than VKAs, managing patients who are bleeding and or requiring urgent surgery or interventions continue to pose a challenge to clinicians. Idarucizumab, a specific antidote for dabigatran, has been effectively studied and reported and is widely available; however, dabigatran use is a small percentage of the NOACs market in most countries. The extensive and growing use of FXa inhibitors focuses on this important management strategy. Andexanet alfa is undergoing additional potential evaluation in traumatic injury and surgery; however, its cost, limited duration of action, and availability pose limitations for clinicians. As a result, the off-label use and role of PCCs for bleeding management in traumatic injury, surgery, and NOAC reversal continue to be reported and studied and represent a potential therapeutic option although off label, and will provide clinical guidelines for use. Although the use of the specific reversal strategies or antidotes will continue to evolve, current recommendations for their use are reported. In early development is also a monoclonal antibody Fab fragment to reverse ticagrelor.

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Part VIII

Future Directions

40 Randomized Controlled Trials: Informing Clinical Practice for Traumatically Injured Patients

Katherine M. Reitz, Andrew D. Althouse, and Jason L. Sperry

Abbreviations

COMBAT	Control of Major Bleeding After Trauma
CRASH	Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage
DSMB	Data and Safety Monitoring Boards
IRB	Institutional Review Boards
PAMPer	Prehospital Air Medical Plasma
PROMMTT	PRospective, Observational, Multicenter, Major Trauma Transfusion
PROPPR	Pragmatic, Randomized Optimal Platelet and Plasma Ratios
RCT	Randomized controlled trial
STAAMP	Study of Tranexamic Acid during Air Medical Prehospital
TXA	Tranexamic acid

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Introduction to Randomized Controlled Trials in Traumatically Injured Patients

Randomized controlled trials (RCTs) first became a prominent tool for testing medical therapies in the mid-twentieth century. As the "gold standard" for evaluating and comparing therapies, the results of a trial may be used to inform clinical practice, potentially improving outcomes for future patients. The act of assigning treatments at random minimizes any systematic differences between the treated groups, allowing us to make probabilistic statements about the observed difference in study outcomes [1]. However, while randomization minimizes systematic bias in treatment group allocation, there are many other threats to a trial's integrity that must be appropriately planned for and addressed in the study design [2]. Therefore, the RCT requires careful planning to ensure generalizable, high-quality results that may improve the outcomes of patients suffering from traumatic events.

Over the last few decades, multiple key randomized trials have dramatically changed the resuscitation of the traumatically injured patient. A select few will be reviewed here (Fig. 40.1). In the 1990s, the effects of resuscitative therapies were first evaluated in traumatically injured patients [3, 4]. In 1994, Bickell et al. evaluated the use of intravenous crystalloid in those with

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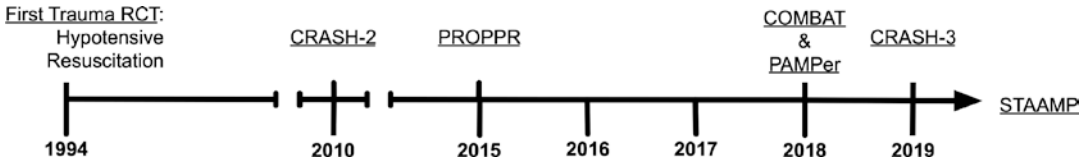


Fig. 40.1 Timeline of key trauma randomized controlled trials

penetrating injuries. This work established the utility of permissive hypotension, in that minimizing intravenous crystalloid infusions and, therefore hemodilution, reduced the coagulopathy of hemorrhage and trauma, improving overall survival [4]. The evaluation of alternative resuscitation techniques including coagulation factors [5–8], human polymerized hemoglobin [9], and blood products [10–14] dominates the next two decades of civilian trials in the traumatically injured.

Randomized Controlled Trials: Fibrinolysis Inhibitors in Trauma

Significant blood loss, even without iatrogenic hemodilution with crystalloid, presents an extreme challenge to the coagulation system. Part of the physiologic response to trauma is the stimulation of fibrinolysis which can pathologically progress to hyper-fibrinolysis and trauma-induced coagulopathy. Multiple trials have evaluated the effectiveness and safety of adjunctive therapies to control bleeding including tranexamic acid (TXA) [5, 6] and recombinant factor VIIa [7] in traumatically injured patients. The Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage (CRASH-2) [6] trial was published in 2010. This international RCT included over 20,000 adults with clinically significant bleeding. Patients were randomized to TXA or placebo in 40 countries with <2% receiving care in hospitals with a developed trauma system. The authors reported that those receiving this fibrinolysis inhibitor experienced a reduction in both 28-day mortality (1.5%) and death from hemorrhage (0.8%) without a difference in vascular occlusive events.

Building on the knowledge from the CRASH-2 trial, the CRASH-3 trial evaluated the effect of TXA administration within 3 hours of a traumatic brain injury in over 12,000 patients in 29 countries [5]. Early TXA treatment most substantially decreased the rate of 28-day head injury-related death in those with mild to moderate traumatic brain injury.

Ongoing concerns as to the ideal treatment timing and optimal protocol have limited the ubiquitous clinical adoption of TXA in those with hemorrhagic shock in civilian trauma centers in the United States. The data from the CRASH studies in combination with retrospective military studies (i.e., MATTERs and MATTERs II) [15, 16] led to the Study of Tranexamic Acid during Air Medical Prehospital transport (STAAMP) trial [8]. STAAMP, which has just completed patient enrollment, is designed to address the use of TXA in civilian trauma.

Randomized Controlled Trials: Blood Product Administration in Trauma

Military trauma experiences showed damage control resuscitation, or early administration of intravenous blood products to maintain perfusion and avoiding hemodilution, improved outcomes for traumatically injured patients. In 2013, the PRospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) study demonstrated an association between early plasma and platelet administration with improved survival [14].

The effectiveness of higher ratios of plasma and platelet administration was first tested in Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) [13]. This multisite

study randomized 680 hospitalized patients in parallel groups to 1:1:1 vs. 1:1:2 (plasma:platelet:packed red blood cells) to evaluate 30-day mortality. Although no significant difference in the primary outcome of 30-day mortality was detected, PROPPR established high ratios of in-hospital plasma and platelet administration were safe. Further, those receiving the balanced 1:1:1 resuscitation strategies achieved more homeostasis and less patients died of exsanguination. This work established balanced resuscitation as the in-hospital standard of care in those traumatically injured and at risk of hemorrhagic shock.

Two decades of work established the combination of permissive hypotension and balanced blood product administration, termed damage control resuscitation, as the standard of care for traumatically injured patients at risk of hemorrhage and trauma-induced coagulopathy. The majority of deaths due to hemorrhagic trauma continued to occur within the first hour of hospital arrival [12]. These data highlight the importance of providing lifesaving therapies as close to the onset of injury as possible. The Department of Defense therefore funded two, synchronized randomized controlled trials: Control of Major Bleeding After Trauma (COMBAT) [11] and Prehospital Air Medical Plasma (PAMPer) [10]. Both trials randomized hypotensive, traumatically injured patients to prehospital plasma administration or either prehospital crystalloid (COMBAT) or current standard of care (PAMPer) evaluating the primary outcome of 28-day mortality. COMBAT, a single, urban center trial randomized 144 patients transported by ground to the trauma center. PAMPer, a multi-institutional trial, clustered randomization at the level of the individual airbases and enrolled 501 patients transported by helicopter to a trauma center. COMBAT confirmed the safety of prehospital plasma administration in those rapidly transported to a single urban center, but no survival benefit was observed. The primary analysis from the PAMPer trial again confirmed safety yet also showed a significant reduction in 30-day mortality. In combination, the data from both trials and the associated robust a priori secondary analyses, afforded by the synchronized trial designs, established the effectiveness of prehospital plasma administration to those traumatically injured with hemorrhagic shock with prolonged transport time [17], concurrent prehospital packed red blood cell administration [18], and in those with a blunt mechanism injury [19].

Overall, these studies work to improve the outcomes of traumatically injured patients at risk for hemorrhagic shock and trauma-induced coagulopathy. Each design effectively tested hypotheses in a specific patient population and transparently reported the study design and results to inform mainstream clinical practice.

Ethical Considerations in Randomized Controlled Trials

Patients participating in clinical trials place enormous trust in the investigators, who are often also their medical providers. These investigators must protect the interest of enrolled patients, who have no guarantee of direct personal benefit. By design, a group of patients may be assigned to receive an inferior treatment, yet prior to the completion of the trial the relative superiority or inferiority of the treatments is unknown. *Equipoise*, the concept that the superiority of one treatment versus an alternative is collectively unknown, is a crucial pillar of an ethically conducted RCT [20]. Although this contemporary concept may seem simple and obvious, this principal has not always been upheld. A full exploration of the history of human subjects research is beyond the scope of this chapter; however, it is important to highlight that there are historical studies where patients were unwillingly and/or unknowingly forced to participate in clinical research without established safety, scientific rigor, or equipoise. This history led to the development of regulatory groups including, but not limited to, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and local Institutional Review Boards (IRB). These groups evaluate and monitor human subjects research assuring Good Clinical Practice.

Good Clinical Practices includes providing *informed consent*, allowing all participants to understand the potential risks and benefits of participating in clinical research as completely as possible. Additional oversight is necessary for vulnerable patient populations who may not be able to provide informed consent including children, pregnant women, the elderly, prisoners, and those with limited intellectual capabilities. *Respect for patient* participation and the subjects includes maintaining patient privacy, allowing enrolled patients to change their mind and withdraw from the trial without incurring a preserved or real penalty, and the results must be made available to participants. Additionally, clinical researchers must test only those questions with *scientific value*, deriving useful knowledge, and *scientific validity*, by creating a study protocol, statistical design, and scientific reporting answering these important questions. The IRB helps to ensure all clinical research has these features and mandates that all prospective clinical trials have patient safety and protocol adherence monitored by a *Data and Safety Monitoring Board* (DSMB). Special ethical considerations for RCTs in the emergency setting will be discussed below.

Transparency in the a priori development, analysis, and reporting of an RCT is expected. In 1994, a formal list of 21 items was first published as the *CONSORT statement*, mandating that the authors of published work must provide enough information to the reader to know how the trial was performed [21]. Since this initial report, multiple updates and expansions for specific trial designs have been published. Further, in 2000 the US Congress authorized the creation of *ClinicalTrials.gov*, a registry to provide information about and access to RCT for those with serious medical conditions. By 2017, the Food and Drug Administration Amendments Act expanded the use of this website by mandating trial registration [22]. These tools both maintain the ethical obligation to make human experimental research findings publicly available and promote *internal validity*, or confidence that the observed results represent the truth in the population studied, and an accurate assessment of *external validity*, or extent to which the results can be applied or generalized into clinical practice or a general patient population [23].

Special Considerations in Trauma Research

Trials evaluating the effect of therapeutic interventions in patients traumatically injured require a *pragmatic* or practical application to the real-world acuity of trauma, protocol, and analysis plan. Traumatic injury is unpredictable leaving patients in the hands of those who are willing and able to care for them. In this emergency setting, patients and their families may not be able and/or available to provide informed consent at the time of potential RCT enrollment. Yet, in a well-designed RCT with equipoise, the effectiveness of the treatments under evaluation are not established. Immediately excluding those patients or surrogates who cannot provide timely informed consent limits the *generalizability*, or broad applicability, of the trial results.

Randomized controlled trials completed in the emergent setting therefore often utilize the regulatory tool of Exemption From Informed Consent for emergency research, also knonw as EFIC [24]. This feature of RCT design is critical to the evaluation of therapies for traumatically injured patients, but also must be appropriately scrutinized in each context by researchers, the Food and Drug Administration, the IRB, community groups, and potential patients. This policy requires that all trial proceedings be disclosed to community members who may potentially be enrolled in the trial. Disclosure includes community focus groups, community-based advertising, and for the principal investigator to make themselves available for community and potential patients' questions and concerns. This allows potential patients the opportunity to opt out of a trial (e.g., wear medical wrist bands excluding them from trial inclusion) and disenroll without penalty following their ability to provide informed consent. In theory, this allows all potential patients an opportunity to disenroll prior to

meeting all inclusion criteria and no exclusion criteria if a traumatic event is to occur. Admittedly, executing this is very challenging in practice and requires great effort on the part of the investigators and the study team to achieve an acceptable level of community consent.

Trial Protocol and Statistical Analysis Plan

The researchers involved in the development of an RCT have the opportunity to generate data which can be directly applied to and change clinical practice. Therefore, a scientifically valid trial protocol and statistical analysis plan must be carefully designed to test the desired hypothesis and generate data with the maximum scientific value.

The development of safe therapeutic interventions often requires multiple rounds of evaluation that are broadly classified into *four phases* (Table 40.1). These designations were initially created for the development of oncologic pharmaceuticals, yet this terminology has been adapted to describe many RCTs in many disciplines including trauma. Stated simply, phase I and II trials are an early evaluation of the intervention's safety and feasibility in humans, phase III trials compare the intervention to alternatives, and phase IV trials are late postmarketing surveillance of the intervention.

Most studies described in the introduction of this chapter are best characterized as phase III trials, evaluating intervention effectiveness to inform standards of clinical care with clinically relevant and *patient-centered outcomes* including events important to patients and their families (i.e., hospital length of stay, hospital readmission, or death). However, studies that precede the definitive, or pivotal, phase III trial are also vital to the advancement of clinical research. Pilot and feasibility studies may be performed to allow investigators to assess the trial team's ability to enroll and retain patients, get feedback from study personnel and/or participants about study procedures (i.e., *stakeholder engagement*), and refine the study intervention and follow-up protocols. These preliminary pilot and feasibility studies may take the form of phase I or phase II trials. Historically, a phase I trial in which an intervention is first evaluated in humans to assess safety in a very short-term period; often, these studies will include only a few patients (typically fewer than 20) and may not have a control group. A phase II trial typically includes a few dozen participants, assessing the intervention dosing and biologic activity, by evaluating the effect on a *surrogate outcome* including an indirect measure of clinical events, including systemic inflammatory markers, laboratory coagulation parameters, and/or units of blood product transfused.

In some cases, preliminary RCTs do not have a clear analogue to the "phase" designations. For example, a study evaluating provider and patient involvement in a study protocol that has established safety and dosages is neither a phase I, II, nor III trial. In this case a *pilot and feasibility study* often serves the role of the phase I/II study, with objectives to assess the feasibility of implementing the study protocol including number of available patients meeting the stated inclusion

Table 40.1 Randomized controlled trial phase

Trial phase	Trial focus	Dosing	Approximate sample size	Primary outcome
I	Safety, dose selection	Ascending dosages	<20	Adverse events or pharmokinetics
II	Safety, feasibility, short-term efficacy	Therapeutic dosages	10–100	Short-term or physician-centered (surrogate) outcomes
III	Efficacy or effectiveness		100–2000	Long-term or patient-centered outcomes
IV	Postmarket surveillance effectiveness and safety		All seeking treatment	Uncommon or long-term adverse events

criteria, potential recruitment locations, and the ability to complete all study procedures. This information can be used to inform the analogous phase III trial design including the trial protocol and analysis plan which is directly informing clinical care.

For example, the ideal prehospital resuscitation fluid in those traumatically injured with hemorrhagic shock at risk of trauma-induced coagulopathy is an active area of research. In the military setting, transfusion of blood type-specific fresh whole blood, known as "walking blood banks," are are associated with hemostatic and survival benefits [25]. However, fresh and type-specific whole blood transfusions are not readily available in a civilian trauma center. Therefore, two pilot and feasibility studies were conducted. Cotton et al. established the safety and potential decreased need of overall transfusion requirement in those traumatically injured receiving modified whole blood when compared to component therapy (i.e., packed red blood cells, plasma, and platelets) [26]. Yazer et al. evaluated the safety and protocol for providing up to two units of uncrossmatched, leukoreduced group O-positive whole blood at a single center in traumatically injured patients with hemorrhagic shock [27]. This important work has set the stage for additional RCTs to evaluate the effect of prehospital whole blood resuscitation on 28-day mortality in those traumatically injured (PPOWER, ClinicalTrials.gov Identifier: NCT03477006).

Patient Selection

Proper patient selection through the development of *inclusion and exclusion criteria* is important for any RCT. In the context of trauma research, consider a trial designed to evaluate the effect of a new treatment to minimize trauma-induced coagulopathy in those with hemorrhagic shock. If the trial design includes all hospital trauma activations (i.e., level I and II traumas), those enrolled will be a very heterogeneous group of patients, ranging from patients who experienced a fall from standing or isolated extremity stab wounds to victims of high-speed car accidents or multiple gunshot wounds. Therefore, the measured effect of this treatment, intended for those at risk of trauma-induced coagulopathy, may not be fully reflected when summed across this heterogenous group.

The need to identify and evaluate outcomes in this specific patient population was used in the first published trauma RCT by Bickell et al. in 1994 [4]. Adult patients with penetrating torso trauma and a blood pressure of ≤90 mmHg were randomized to either immediate resuscitation (i.e., intravenous crystalloid en route to the trauma center and prior to surgical intervention) or delayed resuscitation to the time of the surgical evaluation. Using this carefully selected patient population, only those with hemorrhagic shock from penetrating injury, the results challenged traditional real-world clinical practices establishing the effectiveness of permissive hypotension.

Mirroring the importance of identifying the group of patients most likely to benefit from the therapy (*inclusion criteria*) is identifying patients for which the therapy of interest may cause harm or in whom the treatment is likely to be futile (*exclusion criteria*). In the aforementioned 1994 trial, the trialists appropriately excluded pregnant women for which the physiology of pregnancy and the mechanical force of the gravid uterus on blood returned to the heart is not fully understood or appreciated. The concept of maintaining patient safety, through adequate exclusion criteria, must always be accounted for and continues to be respected in the contemporary trauma RCTs including PAMPer and COMBAT. These synchronized trial protocols included hemodynamically unstable patients and again excluded pregnant patients in addition to those who cannot (i.e., extremes of age and prisoners) or unlikely to (i.e., family objection) provide informed consent, with mechanisms of injury unlikely to generate significant blunt or penetrating trauma (i.e., fall from standing) or unlikely to survive even with maximal medical care (i.e., penetrating injuries to the brain and/or traumatic cardiac arrest >5 minutes). Therefore, as established from the earliest trauma RCT, traumatically injured

patients at the greatest risk of hemorrhagic shock and the associated trauma-induced coagulopathy are included while excluding patients that (i) may be at increased risk of harm by the treatment or (ii) for whom treatment is likely to be futile.

Outcome of Interest

When designing a trial, the *primary outcome*, or the clinical event for which the trial intervention is intended to improve, drives the statistical analysis plan. Additional *secondary outcomes* both support the primary hypothesis and can be exploratory, generating additional hypotheses. As discussed above, the outcomes of interest for a study are dependent on the study goals (Table 40.1).

In early phase and pilot or feasibility trials, the primary outcome is often a measure of expected adverse events or safety, protocol feasibility or the percentage of the protocol completed and data collected, or sometimes a surrogate outcome. A surrogate outcome may be used to establish that the therapy is biologically active and/or potentially beneficial in a preliminary trial without requiring the sample size needed to conclusively establish benefit on a clinical endpoint. However, late phase trials (phase III) designed to guide clinical practice should use *patient-centered outcomes*, or clinical events important to patients and their families, including death, hospital length of stay, or hospital discharge location (i.e., nursing facilities vs. home). Approximately 20–30% of those who are traumatically injured with hemorrhagic shock die within 28 days. Therefore, 28- or 30-day mortality is commonly used as primary, patient-centered outcome.

Treatment Allocation and Masking

The goal of random treatment allocation is to minimize *systematic error*, thereby allowing the statistical analysis to quantify error attributable to chance [20]. In many cases, *allocation concealment* is a critical design feature, meaning that the next treatment assignment in a trial is unknown to the study team. This is distinct from *blinding*, in which the patient and/or study team does not know the treatment assignment. Allocation concealment is necessary to prevent *selection bias*, where the study team preferentially includes (or excludes) patients based on knowledge of what they would be assigned to receive; for example, if the patient has a very poor prognosis and the study team knows that the next assignment is for the experimental therapy, this could influence their decision to enroll the participant in the trial. This would compromise the intent of randomization and could bias the results if it creates a systematic difference between the treatment groups. In contrast, blinding is necessary to minimize other biases. Some examples include *performance bias*, or differences in how the case proceeds due to knowledge of the intervention, and *ascertainment bias*, or systematic data collection differences between groups [28].

In some cases, blinding the treatment assignment may be impractical or impossible. Notably, the blinding is most important to reduce trial bias when study outcomes are subjectively obsessed as opposed to completely objective. Consider a patient and study team who knows the patient received the experimental therapy. If the study outcome is their pain, rated on a scale from 1 to 10, the patient and the provider may be either intentionally or unintentionally motivated to report and record lower level of pain. However, knowledge of the treatment is less likely to alter the rate of objective outcomes, such as patient mortality.

Randomization

There are a variety of randomization techniques which can be used based upon the disease being studied, the logistics of treatment administration, and the associated analysis plan (Fig. 40.2). *Simple randomization* applies treatments (e.g., saline or TXA) at random for each successive assignment with no regard to those treatment assignments already made. Although this is, by

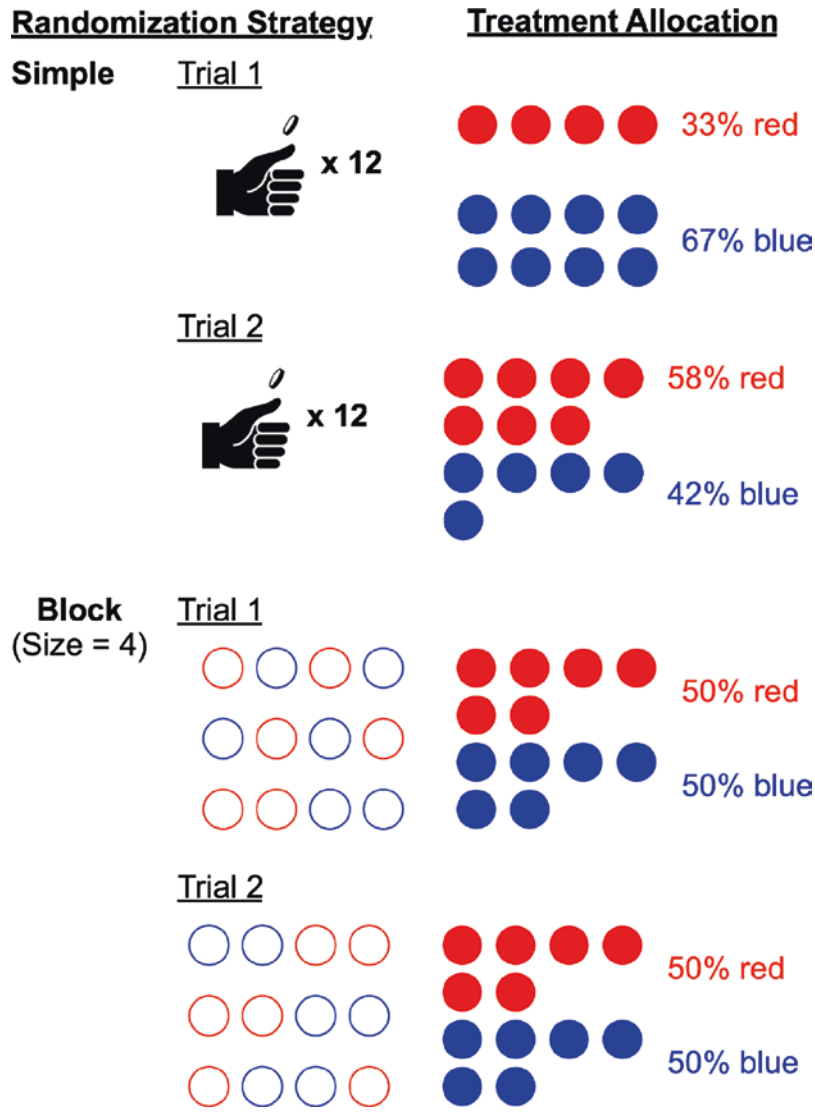


Fig. 40.2 Randomization strategies. In a simple randomization strategy, the flip of a coin occurs when each patient is randomized. The treatment allocation (i.e., red or blue) may therefore not be equally distributed in each trial. Block randomization creates groups (i.e., blocks of four) in which 50% of patients are allocated red and 50% blue guaranteeing balanced treatment allocation for each trial

definition, the simplest approach, it may result in an imbalanced number of patients assigned to treatments (e.g., at random, 30% of patients receiving placebo and 70% TXA), which is generally undesirable due to the loss of statistical power (the ability of the trial to detect a true difference between the treatments). *Block randomization* may be used to limit or constrain the differences in the number of patients randomized to each treatment group throughout the trial. Within each block (usually 4–8 patients), half of patients are randomizing to placebo and half to TXA, ensuring that the number assigned to each treatment can never exceed half of the block size (e.g., a two-arm trial, placebo vs. TXA, with blocks of size 8 can never have a difference in the number assigned to the respective arms greater than four patients), resulting in approximately 50% assigned to each group in a two-group design.

In order to evenly distribute clinically important prognostic factors across treatments, randomization can be *stratified*. For example, if the *treatment effect* (i.e., outcome differences between treatment groups assumed to be secondary to the studied therapy) is expected to be sig-

nificantly different between patients with blunt and penetrating injury, stratifying the randomization by mechanism of injury can balance the distribution of treatments across both subgroups. This balance allows for variability within the trial to be accounted for, therefore improving the efficiency of the trial by maximizing the ability to detect a treatment effect at a given sample size.

Most often, randomization is completed at the level of the patient (e.g., PROPPR, CRASH-2, CRASH-3, COMBAT). Meaning, when a patient is enrolled in a study, their assigned treatment is specific to them. However, *cluster randomization* can also be completed at the level of those providing the intervention and similarly and can be simple, block, or stratified. Overall, this technique is most commonly used when the treatment is expected to affect or be saturated through an entire group of providers. For example, randomizing each patient admitted to a trauma service to either receive a provider-based educational intervention on gun violence or no intervention (i.e., control group) is unlikely to show an effect. Due to participation in the trial, providers are especially cognizant of gun violence and during routine clinical care may use these education tools in the control group as well as the intervention group. This is known as *treatment crossover* and it diminishes the measurable effect of a therapy.

Cluster randomization may also be used for logistical reasons that mirror how an intervention would be implemented outside of an RCT protocol. In the acute, prehospital care of traumatically injured patients, an effective protocol needs to efficiently and easily be implemented. In PAMPer, randomization occurred monthly at the level of the helicopter base. This allowed the treatments to be provided to a geographically diverse set of patients while effectively allocating the limited plasma resource and minimizing waste. Importantly, when completing a cluster RCT, one must account for the effect of clustering, wherein baseline factors and outcomes within a cluster are likely more strongly correlated than patients from different clusters. Although outside of the scope of this chapter, this additional level of complexity needs to be considered in the analysis plan.

Statistical Analysis Plan

A complete description of the statistical analyses of randomized trials is beyond the scope of this book; this chapter will provide general guidance in what to look for in a well-designed trial. Ideally, the statistical analysis plan should be written before any patient enrollment or data collection (i.e., a priori) and shared transparently on ClinicalTrials.gov. The statistical analysis plan may be modified during the course of the trial, but must be finalized prior to database finalizeation and prior to any knowledge of the accrued outcome data. Writing a thorough and transparent analysis plan that is published before the trial outcomes are known ensures that conclusions made about the data are due to the appropriate testing of pre-specified hypotheses, as opposed to retrospectively "hunting" for significant differences after the results are known. This retrospective data evaluation can compromise the integrity of the statistical properties of Type I and Type II error, described below.

A key part of this established, a priori analysis plan is defining primary and secondary outcome variables. For example, PROPPR reported no significant difference in their primary outcome of 28-day mortality. However, the balanced (1:1:1) transfusion group showed improvement in preselected secondary outcomes including 24-hour mortality rates and death from hemorrhage. Readers may generally attach more confidence to secondary outcomes that are pre-specified rather than those retrospectively identified and analyzed, as discussed above. However, the overall Type I error rate is increased when considering more than one outcome, which is why the identification of a primary outcome should reflect the variable with the greatest clinical importance.

Intention to Treat Versus Per Protocol Analyses

In an RCT, perfect adherence to the trial protocol is difficult, and some level of protocol deviation should be expected and accounted for in the analysis plan. For example, one or more patients that

were randomized to the experimental treatment (e.g., 1:1:1, balanced transfusion) may have actually received the control treatment (1:1:2) or vice versa. In the analysis, which treatment group do these patients belong in? This depends on the analysis plan and desired inference. In an *intention to treat* analysis plan, the patient's data are analyzed according to their randomization group regardless of the treatment they actually received. In a *per protocol* analysis plan, the patient's data are analyzed according to the treatment they actually received.

Reporting guidelines suggest all RCTs should be analyzed based on an intention to treat approach [29]. This is chiefly because this strategy represents the real-world effectiveness of the intervention protocol reflecting clinical noncompliance, protocol deviations, and practical challenges with the intervention delivery [30]. However, an intention to treat analysis plan may dilute and underestimate the treatment effect due to these same discrepancies. In such cases, reporting an additional per protocol analysis may be informative. Yet, by definition a per protocol analysis plan compromises the assigned randomization and the results must be interpreted with caution. Specifically, if there is a substantial difference between the intention to treat and the per protocol treatment effect, careful investigation is required to determine the potentials reasons for the discrepancy including the following:

- Were there fundamental baseline differences in those who were included in the intention to treat analysis plan when compared to those in the per protocol analysis?
- Were there systematic differences or specific reasons that enrolled patients did not receive the assigned treatment?

For example, consider again a multi-institutional RCT evaluating a new treatment when compared to placebo administered in the emergency department hypothesized to improve 28-day mortality in traumatically injured patients at risk of trauma-induced coagulopathy. In the intention to treat analysis, including 100% of randomized patients, there are no statistically significant differences in the primary outcome. Yet, in the per protocol analysis, including only 80% of randomized patients, there is a statistically significant difference in the primary outcome. Upon further evaluation, nearly all of the patients who were excluded from the per protocol analysis were enrolled at one specific institution with a high rate of both protocol deviations and prehospital administration of packed red blood cells. Were the protocol deviations because patients received prehospital blood? Is there then no significant treatment effect in those who receive prehospital blood? Or, were the protocol deviations because those patients who received prehospital blood were also rapidly hemorrhaging and therefore more critically ill? Is there then no significant treatment effect in critically ill patients with significant hemorrhage? As exemplified here, while the per protocol analysis can provide interesting information and even a positive trial result, a cause and effect relationship between the treatment and outcomes must be interpreted cautiously.

Statistical Properties of Study Outcomes

In general, most study outcomes will fall into one of the following broadly defined measurement categories: (i) a continuous measure (e.g., hemoglobin, serum creatinine, body weight, etc.) which has an infinite number of possible values; (ii) a count measure which includes both categorical measures (e.g., Glasgow Coma Scale, mechanism of injury, race, etc.) which take on a limited number assignments or binary measure (e.g., intubation, intensive care admission, in-hospital survival, etc.) which represent the answer to a yes/no question; or (iii) a time to event calculated as either the days from randomization to death or from randomization to study follow-up completion. In acute settings, the benefits of time-to-event analyses, as opposed to just a binary outcome, are minimal since loss to follow-up is typically low. Further, small increases in survival time do not always actually represent a meaningful clinical benefit. Understanding the

key differences among these measures of trial outcomes is key to effectively understanding trial outcomes and communicating with a trial statistical when developing a statistical analysis plan.

Covariate Adjustment

If one or more variables are known to have a strong effect on the study outcome, appropriate regression modeling may be included in the primary analysis plan to adjust for these variables. These adjustments can improve trial efficiency by accounting for explainable outcome variation, thereby reducing variability in the estimate of the treatment effect [31, 32]. Like all aspects of RCT analysis, regression modeling must be decided a priori to ensure adjustments with the model were not made solely to achieve a favorable trial result through the statistical manipulation of data.

Sample Size Calculation

As discussed above, the primary outcome is the backbone of an RCT statistical analysis plan. Due to the high rate of mortality associated with those significantly injured and at risk of trauma-induced coagulopathy, 28- or 30-day mortality is commonly chosen. The *effect size*, or the difference in the primary outcome between treatment groups (i.e., treatment effect), is key to establishing the required sample size for any RCT. In general and with all other factors equal, a larger estimated effect size results in a smaller estimated sample size. Importantly, prior to the trial conclusion and data analysis, the actual effect size or treatment effect is unknown.

The estimated effect size should therefore be determined by the principal of *minimum clinically important difference*, also sometimes referred to as the *smallest effect size of interest* which could allow the RCT results to impact clinical practice. For example, traumatically injured patients at risk of hemorrhagic shock have an estimated 20–30% 28-day mortality rate. Consider two simultaneous RCTs. Trial A was designed to evaluate a 5% reduction in mortality for treatment X when compared to placebo in 100 traumatically injured patients. Trial B was designed to evaluate a 1% reduction in mortality for treatment Y when compared to placebo in 500 traumatically injured patients. After trial completion, each well-designed study concluded both therapy X and Y effectively reduce mortality by 5% and 1%, as estimated. However, the minimal effect size associated with treatment Y is not large enough to effectively warrant clinical adoption, but treatment X becomes fully integrated into clinical care. Therefore, the 1% expected and achieved treatment effect for Y is below the minimum clinically important difference and this trial should not have been completed. Therefore, all RCTs should be designed to have sufficient statistical power if the resulting treatment effect is greater than or equal to the minimum clinically important difference.

Error is another important concept in the calculation of a sample size and RCT design (Fig. 40.3). A *Type I error* means that a trial falsely concludes that a treatment effect is present (i.e., there is a significant difference in outcomes between treatment groups) when the

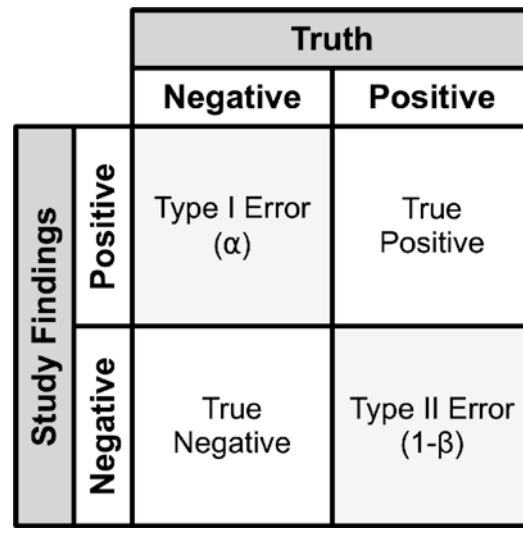


Fig. 40.3 Study result errors. A trial concluding there is a difference between treatment groups when one does not exist is a false-positive result or Type I error (α). Conversely, a trial concluding there is no difference between treatment groups when one does exist is a false-negative result or Type II error ($1-\beta$)

therapy is actually not effective. A *Type II error* occurs when a trial falsely concludes that there is no treatment effect when the therapy actually is effective.

In designing a trial, acceptable levels of Type I and Type II error should be set appropriately for the clinical context. The threshold for Type I error is most commonly set at 5% (or an α level of 0.05), meaning that there is a 5% probability of concluding that a treatment is effective, when it actually is ineffective (false positive). Although uncommon, a higher level of Type I error may be considered if there are no current effective treatments available for a highly morbid disease. In this case, clinicians and patients may be willing to accept a higher rate of a higher chance of concluding a false-positive result in order to allow a treatment for a highly morbid disease to go to market.

The threshold for Type II error is often set at either 10% or 20%, meaning there is a 10% or 20% probability of concluding that a treatment is ineffective, when it is actually effective (false negative). Type II error and statistical power are linearly related (100% – [Type II error] = power), and therefore statistical power is set at 90% (100–10%) or 80% (100–20%), which can also be described as a β of 0.9 or 0.8. At a fixed sample size and estimated effect size, there is an inherent trade-off in the risk of Type I and Type II errors. In general, a larger sample size is required to reduce the risk of Type II error, or the statistical power to find a difference when one does exist, while keeping the risk of Type I error constant. Running trials that are too small to detect a meaningful treatment effect may result in concluding a treatment does not exist (i.e., a negative trial) even for a potentially benefit treatment (Type II error). Again, this reflects the importance of generating the sample size to estimate at least the minimal clinically important difference. Further, artificially increasing the estimated effect size will therefore decrease the calculated sample size; however, this may create trial underpowered to result in a significant difference in treatment groups.

The relationship between effect size, error, and sample size is exemplified in any well-designed RCT. The index trauma RCT by Bickell et al. assumed a 35% mortality rate among patients with penetrating torso injury. They estimated a 10–15% effect size, or a 10-25% reduction in the primary outcome of death, with delayed fluid resuscitation. Using a β of 0.8 (i.e., 80% power), or the percent likelihood of concluding a difference when one does exist, and an α of 0.05, or the percent likelihood of concluding a difference when one does not exist, they planned to enroll 600 patients (Fig. 40.3). If the expected effect size is small, then the sample size required is generally going to be larger. In CRASH-2, the trialists felt that a much smaller 2% effect size, or 2% decrease in the assumed 20% risk of death (the primary outcome), in the TXA group when compared to placebo would be important. Therefore, with 95% power (or β) and an α of 5%, they planned to enroll over 20,000 patients.

Interim Monitoring

All RCTs that are deemed to be more than minimal risk, defined per federal regulation as the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests, should have a DSMB. The DSMB is responsible for periodically reviewing the accumulated study data for participant safety, study conduct, accrued data, and potentially relevant information made available outside of the trial. Like all aspects of RCT design, interim analysis time points and data analysis should be established a priori but can occur emergently if deemed necessary. The DSMB can make recommendations for the study to continue in its current form, continue with modifications, or terminate without further enrollment. Examples of potential reasons for early termination include (1) the trial protocol or treatment is deemed unsafe, (2) the treatment effect is found to be effective prior to enrolling all patients, (3) the likelihood of finding a treatment effect is so small continuing the trial is deemed futile, and (4) new data from an independent study reveals the RCT treatment to have long-term negative health effects.

Conclusion

Randomized controlled trials may be used to inform standards of care and, over time, improve survival in traumatically injured patients at risk of hemorrhagic shock. However, only trials that are carefully and ethically designed, implemented, and analyzed which are adequately and transparently reported should be used to modify norms of clinical practice.

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The Quantra® System and SEER Sonorheometry

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Todd W. Allen, Deborah Winegar, and Francesco Viola

Introduction

The use of whole blood viscoelastic testing (VET) for perioperative and critical bleeding management has seen important evolutionary changes in both technology and clinical application over the last decade. To this effect, the clinical use of VET is approaching the standard of care with increasing numbers of practice guideline recommendations [1–5] in clinical settings where bleeding is a major contributor to poor patient outcomes and increasing costs that are associated with bleeding and blood transfusions. Such clinical areas where bleeding is prevalent are cardiovascular surgery, liver transplantation, obstetric hemorrhage, multilevel spine surgery, and trauma.

While conventional thromboelastometry (ROTEM®, Instrumentation Laboratory, Bedford, MA) and thromboelastography (TEG®, Haemonetics Corp, Braintree, MA) have been the principal VET technologies to date, a novel ultrasound-based VET device named the Quantra® Hemostasis Analyzer (Quantra) (HemoSonics, LLC, Charlottesville, VA) has recently been introduced for clinical use. The Quantra was designed with two main objectives in mind: (1) to further refine VET clot detection methodology and (2) to improve usability factors inherent to other VET platforms that impede broader clinical adoption. The approach taken to improve the usability of VET leverages a novel technology to simplify operator interface, decrease turnaround time to actionable results, and make the interpretation of results easy to understand by clinicians with minimal VET experience.

Measurement Principles

Viscoelasticity refers to a series of properties that characterize how solid materials respond to an applied deformation. When fibrin is produced and polymerized into a three-dimensional structure during coagulation, a viscoelastic solid is formed which exhibits a combination of viscous and elastic behaviors [6]. The viscoelastic properties of the clot evolve dramatically during the process of fibrin network assembly and further change as the platelets aggregate and contract to consolidate the fibrin network. After coagulation, fibrinolysis begins the process of fibrin degradation and the clot returns to a fluid state.

The Quantra uses a patented ultrasound-based technology called Sonic Estimation of Elasticity via Resonance (SEER) sonorheometry that can measure the dynamic evolution of the viscoelastic properties of a clot during the process of

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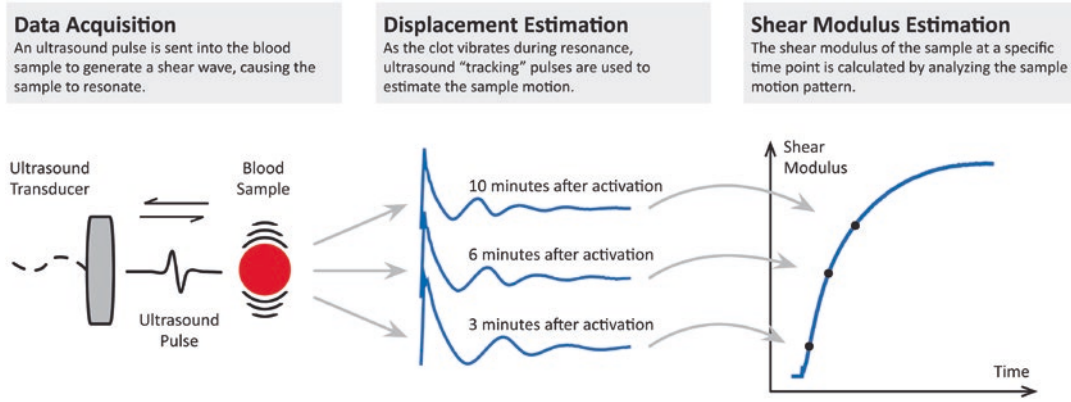


Fig. 41.1 Schematic representation of SEER sonorheometry. The technology is composed of three fundamental steps, as represented by the three panels in this figure. First, a high-energy ultrasound pulse is transmitted in the blood sample to generate a shear wave, causing the sample to resonate (left panel). A series of low-energy ultrasound "tracking" pulses are then sent within the sample and the returning echoes are used to estimate the sample motion (middle panel). The shape of the estimated displacement curve is directly related to the shear modulus of the sample. The time-displacement curve can be compared to theoretical models to determine the actual shear modulus for that specific point in time. This process is repeated every 4 seconds to form a signature curve that shows shear modulus vs time (right panel)

coagulation and fibrinolysis [7, 8]. The technology is shown schematically in Fig. 41.1.

Briefly, SEER sonorheometry uses high-frequency ultrasound pulses to gently nudge the blood sample causing it to vibrate at its resonance frequency (Fig. 41.1, left panel). The sample oscillatory motion induced by the vibration can be measured by tracking time delays of the returning ultrasound echoes (Fig. 41.1, middle panel). The shape and frequency of this vibration are directly related to the viscoelastic properties of the sample. Repeated acquisitions over time form a signature curve that shows the dynamic changes in properties of the sample during coagulation (Fig. 41.1, right panel). Although both the viscous and elastic component of the behavior can be measured, the current implementation of SEER sonorheometry utilizes only the elastic component, the shear modulus of elasticity, which is referred to as the "clot stiffness." From this curve, the start of clot formation, or clot time, and the stiffness of the clot can be directly estimated. These parameters hold combined information about the role of platelets, fibrinogen, and other coagulation factors.

One of the unique characteristics of SEER sonorheometry is that it does not require physical contact with the sample since it is based on the propagation of ultrasound waves. The lack of moving mechanical parts allows small motion to be readily detected. In addition, it means that no disruption is applied to the sample in the early phases of clot formation and assembly. This translates into high sensitivity, especially for weak or soft clots which are those typically associated with perioperative bleeding.

Another important difference from classical VET technologies is that SEER sonorheometry is based on direct measurements of the clot shear modulus, a well-known and objective parameter that describes the elastic properties of a solid material. Shear modulus, expressed in SI (Système Internationale) units of Pascals, represents an absolute measure of clot elasticity which enables Quantra to approximate the relative contributions of fibrinogen and platelets to overall clot stiffness. There is some evidence demonstrating that calculation of the platelet component to clot stiffness should be based on direct measurements of elasticity and not on measurements

of clot amplitude as done with the current VET devices based on thromboelastography or thromboelastometry [9–11].

Quantra Hemostasis Analyzer

The Quantra Analyzer is a stand-alone, automated VET in vitro diagnostic instrument with embedded software (Fig. 41.2). The instrument and the software are a closed system, using only Quantra assay cartridges to perform a specific panel of test measurements. The Quantra Analyzer does not come in direct contact with the blood sample or the reagents at any time during sample preparation and sample testing. No reagents are stored on the instrument. All the aspects of sample testing are automated by the instrument, and a barcode scanner and a touchscreen enable input of operator, sample, and cartridge information and initiation of various activities.

The Quantra Analyzer was designed to operate at the point of care (POC) in critical care environments such as within or near operating rooms, intensive care units, or in the trauma bay and to be used by trained clinical or laboratory personnel. A robust and comprehensive set of internal quality system checks is performed periodically and with every test run to verify proper performance of key operational subsystems. Two levels of external quality control materials are also available to verify the performance of the cartridge test reagents [12].



Fig. 41.2 The Quantra Hemostasis Analyzer (US version shown)



Fig. 41.3 Dials view screens used on the Quantra Hemostasis Analyzer (QPlus). Numerical results of specific parameters are displayed relative to a reference range (green segment on the dial)

The Quantra Analyzer presents test results in three different views: dial results, clot stiffness curves, and trend data. These views are described in more details below (Figs. 41.3 and 41.4). Furthermore, the instrument is also designed with remote viewing capability and can be connected to the hospital laboratory information system (LIS).

Results Views

The primary view screen of the Quantra Analyzer uses a dial for each output parameter, as shown in Fig. 41.3. Each dial shows the reference range, reportable range, parameter abbreviation, and the numerical result for the corresponding parameter. Results that are within the reference range are displayed with a white triangle located on the green portion of the dials. Results that are within the reportable range but outside of the reference range (in green) are signified by an exclamation mark within a yellow circle (as shown in Figs. 41.7 and 41.8).

In addition to the primary dials view, there are two additional screens with supporting informa-

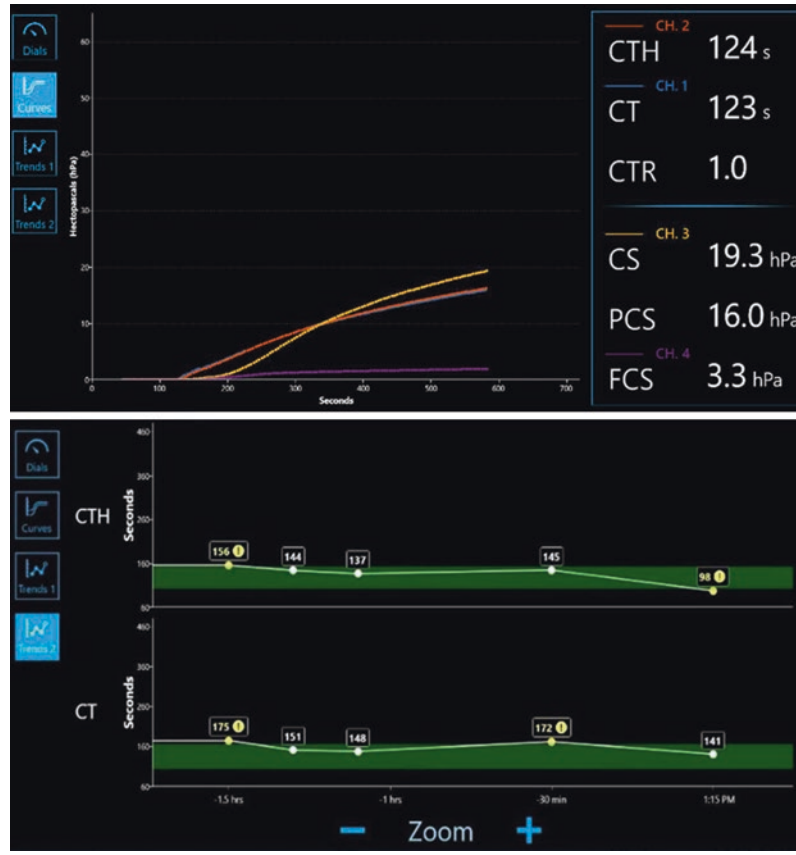


Fig. 41.4 Curves view (top panel) screen and trends view (bottom panel) screen available on the Quantra Hemostasis Analyzer (QPlus)

tion: (1) curves view and (2) trends view. The curves view screen (Fig. 41.4, top panel) shows a graphical display of shear modulus measurements that provides the user with additional visualization of the development of stiffness over time. Using the buttons on the left sidebar, a user can toggle between the dials view and the curves view while the test progresses or after the test is completed. Numerical value results from the dials screen are posted in the right sidebar upon test completion. In the trends view screen (Fig. 41.4, bottom panel), the Quantra Analyzer displays numeric results of tests run during the past 48 hours for the patient ID selected.

Quantra Cartridges

The cartridges utilized with the Quantra Analyzer are multichannel, single-use, disposable plastic components. The cartridges have four independent channels, each containing pre-filled lyophilized reagents in the form of beads that enable simultaneous differential testing without the need for any reagent preparation or pipetting. Lyophilization of the reagents provides stability at room temperature.

The cartridge is the only component of the device that is in direct contact with blood. The sample is introduced into the cartridge by attaching a standard evacuated tube with 3.2% sodium citrate and whole blood directly to the cartridge input port. The sample in the tube is automatically drawn into the cartridge via a vented needle assembly as the test starts. Given that ultrasound can easily propagate through plastic components, the cartridges are fully sealed with no blood-air interfaces. This design feature not only mitigates the potential for biohazard spills, but it also provides robustness against interfering environmental factors, i.e., vibration and evaporation. The approved indication for clinical use may vary by

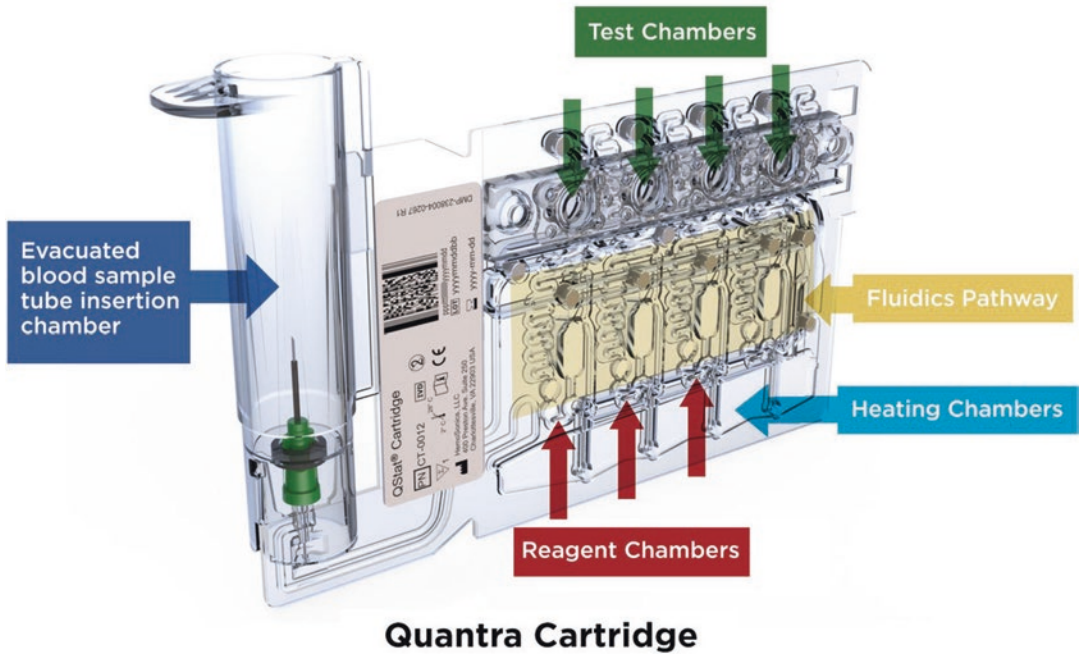


Fig. 41.5 The Quantra Cartridge. The cartridge is a single-use multichannel component that performs four measurements in parallel and forms a closed system. The cartridge includes a safety guard for the insertion of evacuated tubes, a heating chamber to bring the sample to 37 °C, and a series of metering/mixing/test chamber circuits that include the lyophilized test reagents

country. A functional schematic representation of the cartridge is shown in Fig. 41.5.

QPlus® Cartridge

The QPlus Cartridge was designed to evaluate a patient's functional coagulation status in cardiovascular or major orthopedic surgeries before, during, and following the procedure. The cartridge can be stored at room temperature thus making it immediately available for acute bleeding situations without warming or special preparation. The measured parameters include the clot time (CT) with intrinsic activation via kaolin, the heparinase clot time (CTH) via activation with kaolin and in the absence of any heparin influence due to the addition of heparinase I, overall clot stiffness (CS) via extrinsic activation with tissue factor and polybrene to neutralize potential heparin,and fibrinogen contribution to stiffness (FCS) with tissue factor activation in addition to abciximab to block platelets and polybrene to neutralize heparin. Heparinase and polybrene can eliminate any heparin influence up to 6 U/mL. Two additional calculated functional parameters are reported: clot time ratio (CTR), defined as the ratio of CT and CTH, and platelet contribution to stiffness (PCS), defined as the difference between the direct measures of CS and FCS. A detailed description of the QPlus parameters is presented in Table 41.1.

QStat™ Cartridge

The QStat Cartridge was designed to evaluate hemostatic function in trauma, liver transplantation, and other critical care settings. The cartridge, which can be stored at room temperature, measures clot time (CT) with intrinsic activation via kaolin; clot stiffness (CS) via extrinsic activation with tissue factor and polybrene; and fibrinogen contribution to stiffness (FCS) with tissue factor

Table 41.1 Parameter output by the QPlus Cartridge

QPlus parameter	Unit	Description	Measurement	Reference interval[a]
Clot time (CT)	Sec	Clot time in citrated whole blood	Measured with activation of the intrinsic pathway (kaolin)	104–166
Heparinase clot time (CTH)	Sec	Clot time in citrated whole blood with heparin neutralization	Measured with activation of the intrinsic pathway (kaolin) and heparinase	103–153
Clot time ratio (CTR)	N/A	May indicate the prolongation of the intrinsic pathway clot time that is likely due to the influence of unfractionated heparin	Calculated as the ratio of CT and CTH	N/A
Clot stiffness (CS)	hPa	Stiffness of the whole blood clot	Measured with activation of the extrinsic pathway (thromboplastin) and heparin inhibition (polybrene)	13.0–33.2
Fibrinogen contribution to stiffness (FCS)	hPa	Contribution of functional fibrinogen to overall clot stiffness	Measured with activation of the extrinsic pathway (thromboplastin), heparin inhibition (polybrene), and platelet inhibition (abciximab)	1.0–3.7
Platelet contribution to stiffness (PCS)	hPa	Contribution of platelet activity to overall clot stiffness	Calculated by subtracting FCS from CS	11.9–29.8

[a]US Ref Range; healthy subjects 18 years and older

activation in addition to abciximab to block platelets and polybrene. The polybrene can eliminate heparin influence up to 6 U/mL. The QStat Cartridge reports two additional parameters: platelet contribution to stiffness (PCS), defined as the difference between the direct measures of CS and FCS, and clot stability to lysis (CSL) which is defined as the normalized difference of the clot stiffness change after maximum clot stiffness in the absence of tranexamic acid and the corresponding clot stiffness change in the presence of tranexamic acid. Differently from conventional VET devices, the CSL parameter has been uniquely designed to be specific to clot lysis and not influenced by other factors such as clot retraction. A detailed description of the QStat Cartridge parameters is presented in Table 41.2.

Interpretation of Clinical Results

When using the QPlus or QStat Cartridge, the first line of dials reports one or more results that reflect the time of clot initiation (CT, CTH). For these parameters, results within the green reference range are considered to reflect the normal range established in reference range studies; results above the green range are considered as slower clot initiation; and results below the green range are considered quicker clot initiation (Fig. 41.6, top panel).

The second row of dials displayed when using both the QPlus and QStat Cartridge represents results describing clot stiffness parameters (CS, PCS, and FCS). These are measured and reported in hecto-pascals (1 hPa = 100 Pa). Results within the green reference range are considered to reflect the normal range of clot stiffness established in reference range studies, results above the green range are considered as high clot stiffness, and results below the green range are considered lower clot stiffness (Fig. 41.6, bottom panel). The boundaries of the reference range intervals for each parameter should not be interpreted as therapeutic triggers or target values, which will be discussed later in this chapter.

Examples of normal and abnormal results obtained using the QPlus (Panels A, B, C) and QStat (Panel D) Cartridges are shown below in Fig. 41.7.

Table 41.2 Parameter output by the QStat Cartridge

QPlus parameter	Unit	Description	Measurement	Reference interval[a]
Clot time (CT)	Sec	Clot time in citrated whole blood	Measured with activation of the intrinsic pathway (kaolin)	113–164
Clot stiffness (CS)	hPa	Stiffness of the whole blood clot	Measured with activation of the extrinsic pathway (thromboplastin) and heparin inhibition (polybrene)	13.0–33.2
Fibrinogen contribution to stiffness (FCS)	hPa	Contribution of functional fibrinogen to overall clot stiffness	Measured with activation of the extrinsic pathway (thromboplastin), heparin inhibition (polybrene), and platelet inhibition (abciximab)	1.0–3.7
Platelet contribution to stiffness (PCS)	hPa	Contribution of platelet activity to overall clot stiffness	Calculated by subtracting FCS from CS	11.9–29.8
Clot stability to lysis (CSL)	%	Reduction of clot stiffness that is likely due to the influence of fibrinolysis	Calculated by normalizing the rate of stiffness reduction observed with thromboplastin activation with the corresponding change in clot stiffness observed with thromboplastin activation and tranexamic acid	–93–100%

[a]Healthy subjects 18 years and older



Fig. 41.6 Basic interpretation of results relative to the reference interval (green segment). (Top left) Normal CT (clot time) measured in seconds; (Top middle) Slower CT; (Top right) Quicker CT. (Bottom left) Normal CS (clot stiffness) measured in hecto-pascal (hPa); (Bottom middle) Softer or weaker CS; (Bottom right) Stiffer or stronger CS

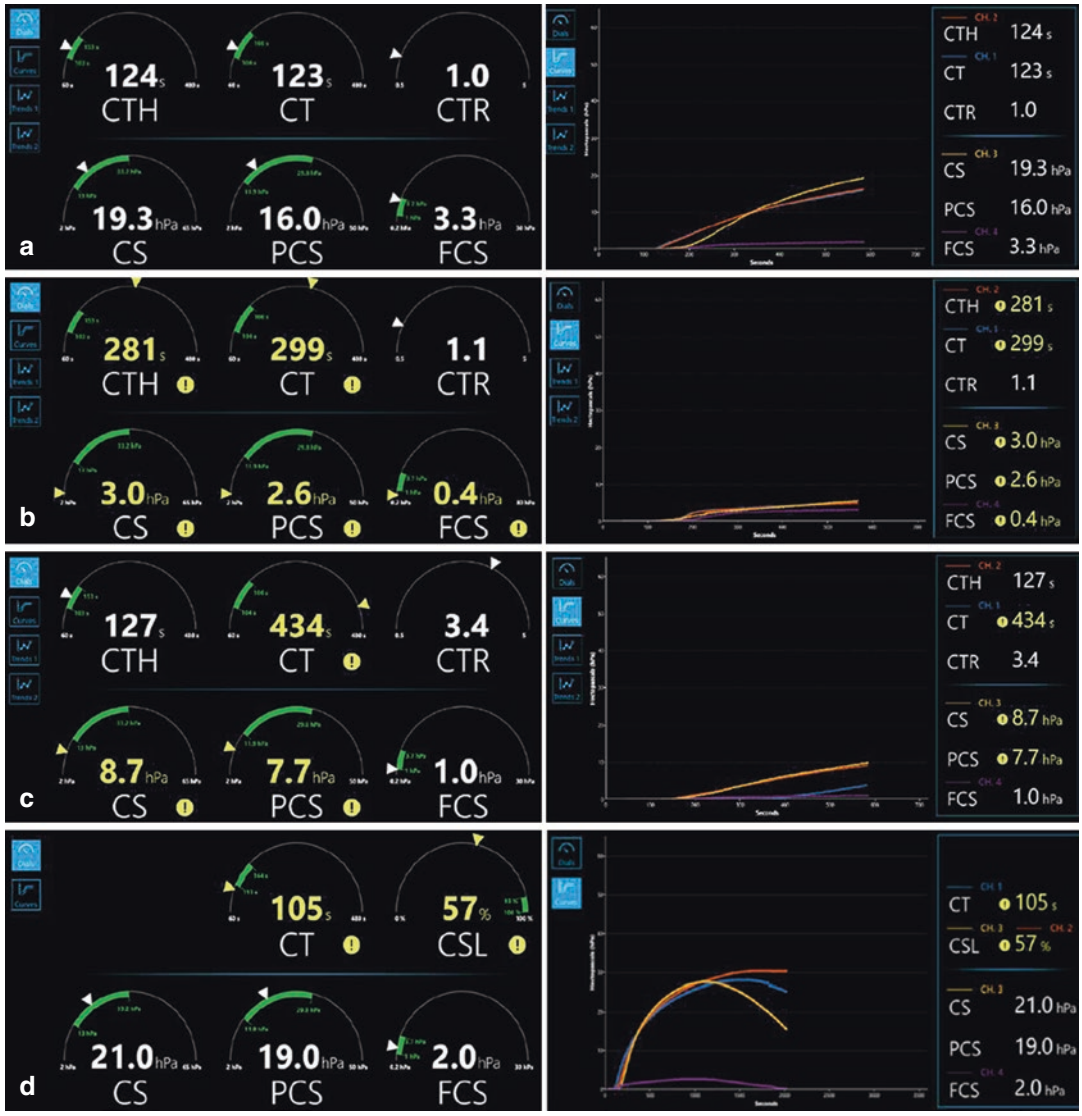


Fig. 41.7 Examples of results on the Quantra QPlus and QStat. (**a**) Normal QPlus results. There is no defect in clot initiation, and CTs are normal. There is no defect in clot stiffness (CS), platelet contribution (PCS), or fibrinogen contribution (FCS). (**b**) Slow clot initiation and low clot stiffness in QPlus. The CT/CTH is delayed without influence from heparin (CTR <1.4). CS is very low indicating an extremely soft clot due to both low platelet (PCS) and fibrinogen (FCS) contributions to clot stiffness. (**c**) Heparin influence and low clot stiffness in QPlus. The CT is significantly delayed and corrected in the CTH and clearly indicated by a CTR of >1.4. The CS is also low due to low platelet (PCS) and borderline low fibrinogen (FCS). (**d**) Quicker clot time with subsequent clot lysis in QStat. The CT is too quick, and the clot lysis is evident by the curve view (right panel) and the CSL parameter of 57% of clot remaining at parameter fulfillment. The FCS curve view can also confirm clot lysis (right panel)

Patient Management Algorithms

VET is often recommended for use in conjunction with POC VET-guided treatment algorithms for the management of acute bleeding in specific clinical settings [1–5], A large amount of literature describes such treatment algorithms in a variety of clinical settings guided by standard lab testing (SLTs), TEG, ROTEM, and other POC-based coagulation tests [13–17]. The outputs

provided by the Quantra System are well-suited to support current recommendations and to provide information that can be readily incorporated in an algorithm to manage trauma-induced coagulopathies. Although the clinical utilization of the Quantra System is limited at the time of this publication given the recent introduction of this platform, potential trigger and therapeutic cutoff values have been derived using results from multiple observational studies that compared the performance of the Quantra System to that of SLTs, TEG, and ROTEM assays [18]. Validation of these cutoff values is underway at several clinical sites.

In keeping with current practice recommended for other VET systems [13–17], treatment guides that utilize data generated by the Quantra System will include (1) therapeutic priority to treat, (2) standardized trigger values, (3) standardized target values, and (4) target-based dosing guides.

Many published treatment algorithms guided by VET take a common approach to prioritize the treatment of bleeding patients [13–17]. This approach is described below in order of priority:

1. Use VET only if clinically significant bleeding is present or if traumatic brain injury is suspected.
2. Optimize base conditions (temp, pH, Hct, Ca^{++}).
3. Consider the clinical need for antifibrinolytic agents.
4. Consider the status of thromboprophylaxis therapy within the clinical setting (unfractionated heparin, low molecular weight heparins, direct oral anticoagulants, etc.).
5. Consider the contribution of substrate to clot stiffness (fibrinogen and platelets).
6. Assess contribution of clotting factors to thrombin generation (clot initiation).

This approach should always accompany knowledge of the clinical context, severity of injury, presence or absence of massive bleeding, and any prehospital treatment that may have occurred prior to testing.

In the clinical context of significant bleeding, *trigger values* represent the threshold at which a decision to treat a specific component deficit should occur, whereas *target values* represent the goal to achieve with specific type and amount of therapy. The therapeutic objective is to match the right type and amount of therapy to the type and degree of component deficit to achieve complete or near cessation of bleeding with the first round of therapy. These trigger and target values may be subject to such variables as the clinical setting (trauma, cardiac surgery, postpartum hemorrhage, etc.) and the severity or type of injury.

An example of a treatment guide for the management of trauma patients using results obtained with Quantra QStat Cartridge developed based on the approaches previously described [13–17] is presented in Fig. 41.8.

Establishing Trigger and Target Values

The relationship between the elasticity-based parameters (CS, FCS, PCS) reported in measurement units of hecto-pascal (hPa) and standard laboratory test parameters (e.g., platelet count and fibrinogen via Clauss methodology) has been established based on regression analysis of data from multicenter observational studies [18]. Further, from these same studies, receiver oerating characteristic (ROC) curve analyses were performed to obtain QPlus Cartridge cutoff values for fibrinogen and platelet contribution to clot stiffness that correspond to common treatment decision-making thresholds. Tables 41.3 and 41.4 present results from ROC curve analyses to assess the ability of Quantra System FCS to predict fibrinogen levels determined by the Clauss assay and to assess the ability of PCS to predict platelet count.

Using similar analyses, the relationship between Quantra FCS and FIBTEM A20 and Quantra CS parameters has been established [18] as shown in Table 41.5.

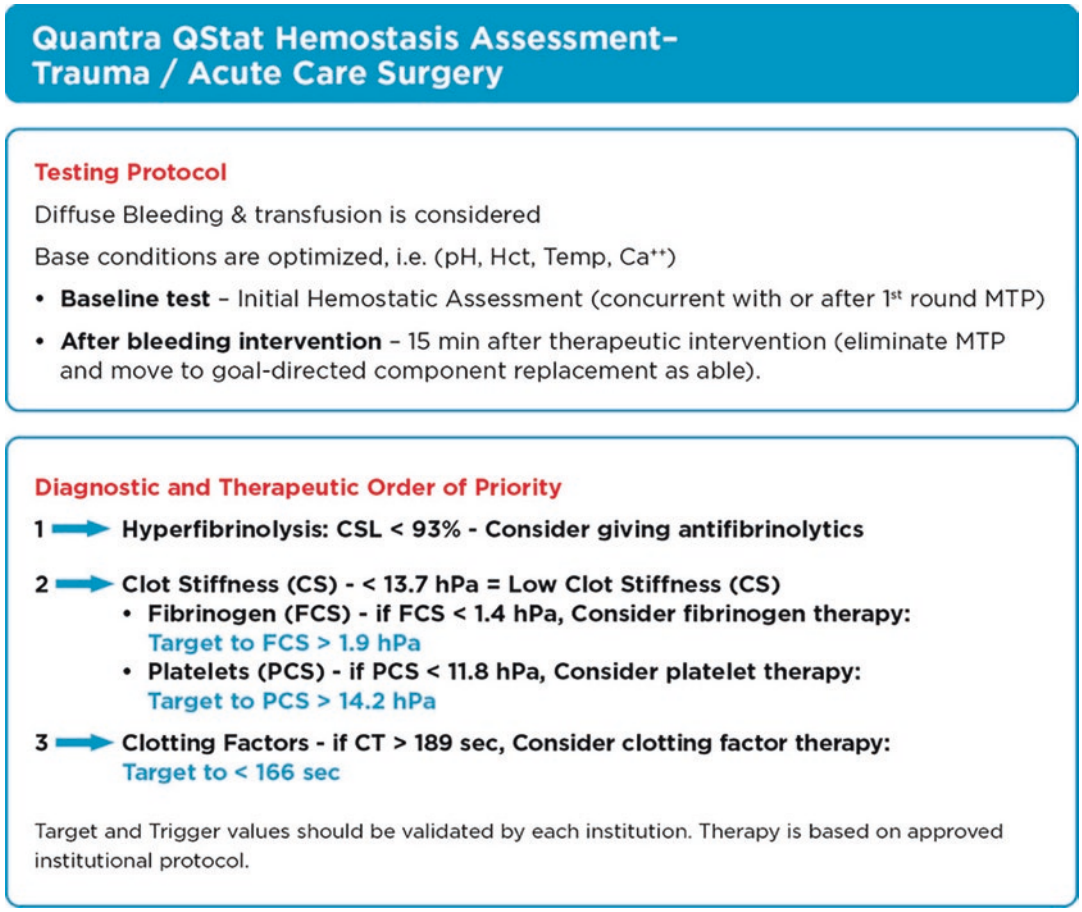


Fig. 41.8 Example of treatment guide based on results of Quantra QStat Cartridge for the management of trauma patients

Table 41.3 ROC curve analysis fibrinogen via Clauss to Quantra FCS parameter

Clauss fibrinogen cutoff value (mg/dL)	QPlus cutoff value FCS (hPa)	N (yes/no)	AUC	Sensitivity	Specificity	NPV
<120 mg/dL	FCS < 1.1 hPa	18/740	0.97	0.90	0.95	0.99
<150 mg/dL	FCS < 1.3 hPa	39/667	0.96	0.91	0.88	0.99
<200 mg/dL	FCS < 1.9 hPa	152/476	0.92	0.86	0.76	0.95

Table 41.4 ROC curve analysis of platelet count to Quantra PCS parameter

Platelet count cutoff value	QPlus cutoff value PCS (hPa)	N (yes/no)	AUC	Sensitivity	Specificity	NPV
<80,000	PCS < 12.1 hPa	29/668	0.92	0.94	0.83	0.99
<100,000	PCS < 14.1 hPa	75/553	0.88	0.89	0.74	0.98
<150,000	PCS < 18.0 hPa	318/323	0.85	0.85	0.71	0.85

Table 41.5 ROC curve analysis of ROTEM parameter values to Quantra parameter values

Model	QPlus cutoff value	N (yes/no)	AUC	Sensitivity	Specificity	NPV
FIBTEM A20 < 8 mm vs FCS	FCS < 1.2 hPa	45/555	0.85	0.67	0.92	0.97
FIBTEM A20 < 10 mm vs FCS	FCS < 1.4 hPa	87/513	0.92	0.77	0.90	0.96
FIBTEM A20 < 12 mm vs FCS	FCS < 1.7 hPa	145/455	0.94	0.88	0.86	0.96
EXTEM A20 < 45 mm vs CS	CS < 10.5 hPa	25/578	0.77	0.64	0.96	0.98
EXTEM A20 < 50 mm vs CS	CS < 13.7 hPa	67/536	0.90	0.84	0.88	0.98
EXTEM A20 < 55 mm vs CS	CS < 15.9 hPa	152/451	0.92	0.88	0.84	0.95

Additional Considerations

As previously described, the Quantra System was purposely designed to leverage a novel technology to increase the usability of VET and improve patient outcomes. This approach focuses on providing reliable information to assess a patient's functional coagulation status and/or hemostatic function in a user-friendly manner. The reported parameters focus on the most common treatable coagulation defect, thus making it easy to match the therapeutic intervention to the specific deficiency. Work is ongoing to assess the need for expanding reportable assay parameters with our existing cartridges or developing new assay panels as dictated by unmet clinical need.

It must be noted that the Quantra System shares some of the same limitations that currently affect the conventional VET systems. The QPlus and QStat Cartridges were not designed to measure the level of inhibition of specific platelet receptor activities resulting from antiplatelet therapies (e.g., clopidogrel and aspirin); therefore, the influence of these therapies on Quantra parameters remains to be determined. Second, Quantra System measurements do not reflect the interactions between blood and the vascular endothelium (e.g., the effects of von Willebrand factor). Although the dynamic interactions between the vasculature and the coagulation/fibrinolytic systems may provide important information about the pathophysiology and treatment of trauma patients, neither the QPlus nor QStat Cartridges were designed to monitor these interactions. Finally, any VET platform, including the Quantra System, should not be used as the sole consideration for diagnosing hemostatic derangement. Other POC and laboratory assays along with a comprehensive clinical assessment and sound clinical judgment should be employed for managing clinically significant bleeding.

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Dried Plasma for Trauma Resuscitation

42

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Introduction

Early plasma transfusion improves survival after trauma and hemorrhagic shock. The administration of plasma for treatment of trauma is based on at least three mechanisms: (a) provision of normal concentrations of coagulation and anticoagulation factors; (b) protection of endothelial glycocalyx and preservation/restoration of endothelial function; and (c) provision/restoration of intravascular volume. Dried plasma offers an additional advantage over fresh frozen plasma or liquid plasma: that of logistics. It does not require storage and cold-chain transportation at −80 C, as does FFP; has a longer expiration date than does liquid plasma; and is of smaller size and weight than either of the other two plasma products. This logistic advantage offers the possible clinical advantage of a more clinically timely administration in civilian and military prehospital environments. The latter is especially important in light of the accumulating evidence of the important efficacy of early transfusion of plasma after trauma and hemorrhage.

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This chapter describes briefly the basis for plasma therapy in trauma; the history of dried plasma, production, and currently available and potential future products; and preclinical and clinical evidence of efficacy and safety.

Importance of Early Plasma Transfusion: Need for Dried Plasma

Hemorrhage is the leading cause of potentially preventable death in military trauma and the second overall cause of death in civilian trauma [1, 2]. Over the past 10 years, the critical role of balanced transfusion for resuscitation has been recognized and is now reflected in clinical practice [3]. In contrast to earlier approaches, which relied heavily on crystalloids and red blood cells (RBC), the more recent emphasis is to include plasma early to achieve a 1:1–1:2 plasma to RBC ratio [4]. While crystalloid-based resuscitation can lead to hemodilution, progressively deranged hemostatic and fibrinolytic mechanisms, and endothelial dysfunction, plasma transfusion restores homeostasis and supports the endothelium. The survival benefit of plasma is most dramatic among patients likely to die as a result of bleeding, within the first 6 hours of injury [5–7].

Recent studies have demonstrated that transfusion often cannot wait until arrival at the trauma center and that early plasma can be lifesaving. The impact of prehospital transfusion on survival

A L G r a w a n y

was evaluated retrospectively in a concurrent cohort study of 502 US combat casualties in Afghanistan between 2012 and 2015. The study included casualties with hemorrhagic shock or multiple amputations who were rescued by MEDEVAC helicopter. The median time from injury to arrival at a surgical capability was 47 minutes. In this severely injured cohort (Injury Severity Score = 29), survival was improved in patients who received prehospital blood product transfusion, compared to those who did not (mortality hazard ratio 0.26 and 0.39, at 24 hours and 30 days, respectively). Initiation of transfusion within 15 minutes of MEDEVAC rescue (median 36 minutes after injury) was associated with improved survival (mortality hazard ratio = 0.17), while delays beyond that eliminated the effect [8].

More recently, two civilian studies of prehospital plasma administration were reported. Sperry et al. [9] conducted a prospective, randomized, multicenter study of over 500 trauma patients with hemorrhagic shock who were transported by helicopter. Patients received standard care en route with or without the addition of 2 units of thawed plasma prior to other resuscitation measures. Median prehospital transport time was 42 minutes. Prehospital administration of plasma resulted in a significantly lower mortality (23.2% versus 33.0%, $p = 0.03$), compared to the standard of care group [9, 10]. In a similar study conducted in a ground ambulance rapid transport system (median prehospital time: 19 minutes), no survival advantage was observed for prehospital plasma [11, 12].

A post hoc analysis of these two clinical trials showed that prehospital plasma provided an overall survival benefit and that the response to plasma was influenced by prehospital transport time. When prehospital transport time (time from arrival on scene to arrival at the trauma center) was longer than 20 minutes, mortality was increased among patients that received crystalloid-based resuscitation (HR 2.12; 95% CI, 1.05–4.30; $p = 0.04$), while increased mortality was not observed in patients in the prehospital plasma group (HR 0.78; 95% CI, 0.40–1.51; $p = 0.46$). When time from injury to arrival on scene was accounted for, the findings suggested that plasma transfusion must begin within approximately 40 minutes from injury [13]. Another report demonstrated that the combination of prehospital red blood cells (RBC) and prehospital plasma was better than either product alone [14]. This chapter will focus on dried plasma.

Taken together, available data suggest that, for patients who cannot reach a trauma center rapidly, prehospital transfusion should be initiated as soon as possible. Even with rapid prehospital transport, prehospital transfusion may be essential, for example, if a patient is bleeding very rapidly or if there has been a longer period from injury until ambulance arrival.

A number of trauma systems have incorporated RBC and/or plasma on their evacuation helicopters [8, 10, 15–22]. However, logistical constraints currently prohibit out-of-hospital transfusion in most civilian ground ambulances and at rural or remote hospitals without blood banks.

Fresh frozen plasma (FFP) must be stored frozen, takes about 30 minutes to thaw using conventional equipment, and then must be stored, refrigerated, and transfused within 5 days. These requirements present logistical challenges, in terms of transport, storage, and inventory management. Establishment of prehospital air medical thawed plasma programs is feasible, but can be costly [23]. Adams et al. analyzed clinical trial data and estimated that the cost of routinely carrying thawed FFP on helicopters is expensive. Delivery to the helicopter base, storage, and inventory management were cost drivers. Only 7% of units was transfused by helicopter crews. Twenty percent was wasted (expired) and 73% was returned to the blood bank. Of the units returned to the blood bank, only 59% was ultimately transfused. To a degree, wastage was dependent on distance of the air medical base from the blood bank; for the most remote bases, plasma was either transfused or allowed to expire. When considering ground ambulances, the challenges become even more significant. A dried plasma, with reduced cold-chain requirements and easy reconstitution, is needed to enable the broader use of plasma in the prehospital setting [23, 24].

Dried Plasma: History

In the 1930s, methods were established to lyophilize plasma [25], and successful infusion was reported in both experimental animals [26, 27] and humans [28]. Large-scale use of dried plasma began in WWII, when millions of units were distributed to Allied Forces worldwide (Table 42.1). Both the British and Americans produced pooled, lyophilized plasma (Fig. 42.1) [29, 30]. The Swedish Defense Force was provided with a spray-dried plasma [31]. These were regarded as efficacious. By the end of the war, it became apparent that hepatitis transmission was a problem [32], as it was with all other blood components [33]. The use of dried plasma continued through the first part of the Korean War. Following unsuccessful attempts to reduce disease transmission by irradiating plasma [34], US Army policy was changed to recommend that plasma be used only in emergencies and when no plasma expander was available. By 1968, dried pooled plasma was essentially abandoned in the United States [35]. The French Military Blood Institute produced dried plasma from 1949 to 1984 and provided nearly 40,000 units to French military forces during the Indochina War [36]. Production was discontinued in 1985, due to risk of HIV transmission. Improved donor screening and the advent of pathogen reduction

Table 42.1 Historical dried plasma development

1930s Plasma lyophilization developed
1940 – Large-scale production of pooled (1000+ U), lyophilized plasma by both the US and British established for wartime use (to meet logistical constraints of whole blood and frozen/liquid plasma)
1941 – Spray-dried plasma produced for the Swedish Defense Department
WWII Production
British produced >500,000 U in WWII
US produced >6,000,000 U in WWII
US/British distributed worldwide
Swedish ~17,000 U for use in Sweden and Finland
1945 – Hepatitis
Hepatitis as a result of plasma transfusion recognized by end of WWII
Believed that benefits outweighed the risk
1945–1952 – Hepatitis
Attempts at pathogen reduction and reducing pool size not successful
Three normal volunteers died in an experimental study – testing program suspended
1953 – Department of the Army (Circular 73) directed that, because of the risk of serum hepatitis, the higher cost, and the need to use it for the production of specific globulins, plasma would not be used "to support blood volume" unless dextran was not available (US)
1953 – Serum albumin replaced plasma as primary resuscitative product for US Forces in Korea
1968 – National Research Council Committee on Plasma and Plasma Substitutes recommends that "the use of whole, pooled human plasma be discouraged and even discontinued unless a clear cut case can be made for its unique requirements" (US)
1985 – The French Military Blood Institute produced dried plasma from 1949 to 1984 and provided over 40,000 units to French military forces during the Indochina War. In 1985, production was discontinued due to risk of HIV infection

Adapted with permission Pusateri et al. [37]

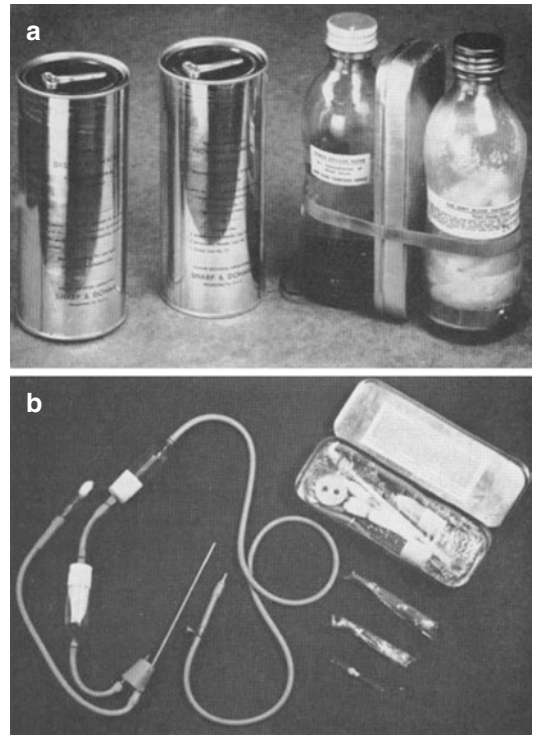


Fig. 42.1 (**a**) British (right) and US Army dried plasma units. (**b**) British dispensing set for plasma. Accessed at (https://upload.wikimedia.org/wikipedia/en/thumb/9/95/Britain_and_us_plasma_packages_wwii.jpg/162px-Britain_and_us_plasma_packages_wwii.jpg, 14 August 2020)

technologies in the 1990s, and their application to blood components, allowed for renewed development and production of safe dried plasma products [37].

Methods of Production

All current dried plasma products incorporate donor screening and testing procedures. Dried plasma can be produced from single units of plasma, or from pooled plasma, in pool sizes ranging from <11 to >1000 (Table 42.2). Pooled products also incorporate pathogen reduction, as an added safety measure. Available products are manufactured in a central facility, while some dried plasmas being developed use a distributed model, producing individual dried plasma units by "bench-top" devices [37, 38]. Available dried plasma products utilize lyophilization, while some products in development use spray-drying. These two processes have fundamental differences. An important concern is that the atomization of liquid plasma to droplets plus the brief exposure to high temperature at the tip of the spray drying nozzle has the potential for damaging proteins (e.g., coagulation and anticoagulation factors) and, in the process, possibly creating neo-antigens that might be clinically important. This has led to more protracted development programs required by some regulatory authorities [38]. The pathogen reduction technologies currently in use for dried plasma production include amotosalen plus UV light treatment and solvent/detergent (S/D) treatment. Each drying and pathogen reduction process has characteristic effects on plasma. However, processes can be optimized to minimize these effects [37–41].

Table 42.2 Characteristics of commercially available dried plasmas

	FLYP	LyoPlas N-w	Bioplasma FDP
Use	1994–present – French Military 2011–present – Civilian (austere) 2018–present – US Military	General population – Germany	General population – South Africa and neighboring countries
Processes	Lyophilized Pooled apheresis FFP <11 donors All volunteer donors Donor screening Testing – disease factors Hemovigilance program 2003 – Leukoreduced 2010 – No HLA Ab+ women 2010 – Amotosalen Pathogen Reduction (PR)	Lyophilized 1990s to 2006: Pooled S/D 2007–present – Single donor Donor screening Hemovigilance program Frozen ≥4 mos for donor retest Leukoreduced No HLA Ab+ women	Lyophilized Pooled (up to 1500 donors) All volunteer donors Donor screening Comprehensive testing Hemovigilance program S/D treatment for PR
Characteristics	Normal factor levels ABO-universal	Normal factor levels ABO type specific	Factor levels: ≥ 0.40 IU ABO-universal plasma
Shelf-life	2 years at room temperature	15 months at 2–25 °C	Store below 25 °C
Reconstitution	<6 min	A few minutes	<10 min
Indication	As sole source of plasma where used	Same as frozen plasma	Where plasma and coagulation factors are required
Safety	No adverse events reported (including TRALI) since 1994 start of hemovigilance program	300,000 U S/D LyoPlas 230,000 U LyoPlas N-w (2007–2013) Hemovigilance program reported no increase incidence of adverse events	Contraindicated: Severe protein S deficiency Hemovigilance program – no increase in adverse events
Efficacy	Clinical use reports support efficacy as part of a 1:1 DCR approach	No restrictions related to clinical efficacy have been identified	No restrictions related to clinical efficacy have been identified

Adapted with permission Pusateri et al. [37]

Therefore, it is important to consider the characteristics of each individual product.

Modern Dried Plasma

Currently Available Dried Plasma Products

By the 1990s, problems with disease transmission were largely addressed by improved donor screening, testing, and pathogen reduction, enabling production of modern dried plasmas. In France, Germany, and South Africa, technological and manufacturing barriers have been resolved, and the consistent production of safe and effective dried plasma has continued for over 20 years [36, 37, 42, 43]. Yet, most countries, including the United States, do not have dried plasma products [24]. Currently, there are three commercially available dried plasmas (Fig. 42.2; Table 42.2).

The French military (French Centre de Transfusion Sanguine des Armées) resumed dried plasma production in 1994, ensuring safety by using a minipool (<11 donors) approach with carefully screened and monitored donors, plasma quarantine and donor retesting, and a robust hemovigilance program (Fig. 42.2a; Table 42.2) [36]. The product excludes plasma from women with antihuman leukocyte antigen antibodies and incorporates pathogen reduction using amotosalen and ultraviolet light (UV) (Cerus Corporation, Concord, CA). In the early 1990s, an S/D pooled lyophilized plasma was developed by the German Red Cross (Fig. 42.2b; Table 42.2). Over 300,000 units of the original product were used through 2006. In 2007, due to risk of transmission of Creutzfeldt-Jakob disease, the German Red Cross switched production to LyoPlas N-w, a single donor, quarantined lyophilized plasma [42]. The National Bioproducts Institute (Pinetown, South Africa) produces Bioplasma FDP, a pooled, S/D, ABO-universal lyophilized



Fig. 42.2 Available dried plasmas: (**a**) *French lyophilized plasma* (*FLYP*), produced by the French Military Blood Institute (Centre de Transfusion Sanguine des Armees (CTSA)); (**b**) *LyoPlas N-w*, produced by the German Red Cross; (**c**) *Bioplasma FDP*, produced by National Bioproducts Institute, Pinetown, South Africa. (Adapted with permission Pusateri et al. [37])

plasma, which has been in use in South Africa since 1996 (Fig. 42.2c; Table 42.2). These products are currently available within each respective country and limited others. French lyophilized plasma is currently available to the US military under an emergency use authorization [38].

Products Currently in Development

A number of companies have been developing dried plasma products for US licensure. Each program is aimed at producing dried plasma that is packaged in a flexible blood bag and has reduced logistical requirements [37, 38]. From 2008 to 2013, HemCon Medical Technologies, Inc. (Portland, Oregon) developed a product that used licensed FFP to produce single-donor lyophilized plasma and thus incorporated the safety measures inherent in FDA licensed FFP. In 2011, the product was successful in a phase I clinical trial [44, 45]. Since 2013, Vascular Solutions (Maple Grove, Minnesota), a subsidiary of Teleflex Corporation, continued this freeze-dried development program for single-donor lyophilized plasma (RePlas) [38]. Entegrion Inc. (Research Triangle Park, NC) has been developing a pooled, S/D, spray-dried, universal plasma (Resusix) since 2008. Plasma is pooled (1000–1500 units), providing the ability to assay and standardize factor levels, which can vary significantly among individual donors [46–48]. The product has completed a phase I clinical trial. Resusix and RePlas are being developed under centralized manufacturing models. Other companies are pursuing a decentralized manufacturing approach. Velico Medical Technologies (Beverly, MA) is developing a spray-drying device and proprietary bag system that will produce licensed, single-donor, spray-dried plasma units [38, 41, 49]. Terumo BCT (Lakewood, Colorado) is developing a single-donor, freeze-dried plasma manufacturing device for use at blood centers [38]. Decentralized manufacturing offers the advantage of the total supply being less vulnerable to manufacturing difficulties while raising concerns regarding quality control and uniformity of product.

Recently, it has become apparent that early plasma transfusion is not only important for humans but also for canine victims of trauma. Guidelines have been established for resuscitation of wounded military working dogs using plasma, and it is expected that these guidelines will be applicable to civilian canine trauma [50, 51]. Recognizing this need, two companies, BodeVet (Rockville, Maryland) and Mantel Technologies, Inc. (Fort Collins, Colorado), are developing canine freeze-dried plasma products for FDA approval.

Clinical Experience with Modern Dried Plasma

Initial experience with modern dried plasma products was in-hospital [42, 52, 53]. Following early case reports on the prehospital use of dried plasma in military operations, military and civilian medical services from a number of countries incorporated dried plasma for point of injury and en route administration in casualties with trauma and hemorrhagic shock [4, 22, 54–60]. Since that time, studies have confirmed both the feasibility and efficacy of the use of this product in the early treatment of patients with traumatic hemorrhage.

French Lyophilized Plasma (FLYP)

The French military first described their use of FLYP in 87 patients in Afghanistan between 2010 and 2011. FLYP transfusion reduced prothrombin time (20.0 ± 9.1–16.7 ± 4.0 s, $p < 0.01$), with no transfusion reactions [36, 52]. More recently, Nguyen et al. [61] conducted a retrospective study at a level 1 trauma center. Among trauma patients who received at least 2 U RBC, the authors compared patients that also received either FFP ($n = 29$) or FLYP ($n = 43$). They found that, while time to receive RBC was similar between groups, patients who were transfused with FLYP received plasma earlier than those that received FFP (15 min versus 95 min, respectively; $p < .01$). These patients also achieved a higher plasma:RBC transfusion ratio during the

first 6 hours and had reduced overall transfusion requirements [61]. FLYP was also studied in a prospective randomized open-label study of severely injured trauma patients who received either up to 4 U of FLYP ($n = 24$) or up to 4 U of FFP ($n = 24$) in combination with up to 4 U of RBC as part of a 1:1 ratio transfusion at a level 1 trauma center. FLYP was immediately reconstituted at the bedside, while FFP was ordered from the blood bank according to standard procedures. Patients treated with FLYP received earlier plasma transfusion and had improved coagulation parameters through the first 6 hours, compared to patients who received FFP, and no adverse events were noted [62]. Reports of the use of FLYP in the prehospital environment have also been promising [60, 63].

LyoPlas N-w

From 2007 to 2011, over 230,000 U of LyoPlas N-w were delivered to German facilities, compared to approximately 343,000 U of FFP [42]. Incidences of transfusion reactions were similar for lyophilized plasma (0.023%) and FFP (0.018%). More recently, Shlaifer et al. reported a retrospective analysis of 109 trauma patients who were treated with prehospital administration of LyoPlas N-w. Prehospital administration was safe (only one mild reaction which stopped when transfusion stopped) and feasible (only five instances of difficulty with administration) [64]. A single-center observational study examined the effect of prehospital administration of LyoPlas N-w by helicopter emergency medical service (HEMS) crews. Patients who received up to 4 U RBC without plasma during a 12-month period were compared to those who received up to 4 U RBC plus up to 4 U LyoPlas N-w over the following 12-month period. After HEMS began carrying LyoPlas, 66 patients received FDP and RBC, 46 received FDP only, and ten received RBC only. The authors concluded that inclusion of prehospital FDP was safe and logistically feasible [65]. Nadler et al. reported a retrospective descriptive case series of 33 pediatric trauma patients with hemorrhagic shock who received LyoPlas N-w at the point of injury, including one who received the plasma via intraosseous infusion. No adverse reactions were noted, and it was concluded that LyoPlas N-w could be used safely and effectively in the prehospital setting for pediatric trauma patients, but that specific treatment protocols should be developed [66]. Benov et al. reported the Israeli Defense Forces' experience with prehospital transfusion of LyoPlas N-w to 75 patients with trauma and hemorrhagic shock, with no adverse reactions or difficulties with administration [67]. Most recently, a retrospective cohort study examined 48 patients that received prehospital LyoPlas N-w compared to 48 patients who did not receive prehospital FDP and showed that INR was improved in patients that received FDP [68]. The suitability of LyoPlas N-w for use in the out-of-hospital environment was confirmed in a recent in vitro study that demonstrated excellent product stability under field conditions [69].

Bioplasma FDP

Bioplasma FDP has been in use in South Africa since 1996, with a strong record of safety [43]. The product is used interchangeably with FFP in all types of patients, depending on availability. In some provinces, such as Kwazulu-Natal, Bioplasma FDP is the primary form of plasma used (Dr. Lee Wallis, "personal communication"). Hemovigilance data collected during the first 10 years of use demonstrated a high degree of safety with only 48 adverse events after transfusion of a total of 372,485 bottles of Bioplasma FDP (1996–2006) [43]. The majority of these adverse events were immunologic/allergic reactions, and the only reported case of transfusion-related acute lung injury was not confirmed. Hemovigilance data also showed that transfusion-related adverse events were reduced with BioPlasma FDP, compared to single-donor FFP in South Africa [43]. A prospective single-blinded consecutive enrollment trial compared the efficacy and safety of Bioplasma FDP ($n = 23$) to non-S/D treated single donor fresh-dried plasma ($n = 20$) in patients requiring

cardiopulmonary bypass [53]. The two plasmas performed similarly, with no differences in coagulation or physiological parameters and no adverse events.

Outlook for Trauma-Induced Coagulopathy: Endotheliopathy, Coagulation, and Fibrinolysis

Trauma-induced coagulopathy occurs within minutes of injury, in approximately 25% of severely injured patients, and is associated with increased mortality [70–72]. Mechanisms are complex and result in imbalances among the procoagulant, anticoagulant, fibrinolytic, and antifibrinolytic systems, leading to multiple phenotypes that can vary based on injury pattern, time after injury, and iatrogenic factors [73–76]. An endotheliopathy of trauma (EOT) also occurs early after injury and contributes to the coagulopathic phenotype [77, 78].

The EOT is characterized by systemic vascular endothelial permeability, coagulation dysfunction, hemodynamic instability, inflammation, and organ injury after hemorrhagic shock [78–80]. Endotheliopathy can occur within minutes of injury and is associated with poor outcomes, including multi-organ failure [77]. Therefore, restoring the endothelium and barrier integrity is integral to further reducing hemorrhage-related morbidity and mortality. Currently available data suggest that plasma is the most promising treatment for EOT, both in terms of efficacy and availability, while other plasma-derived products are also promising [78]. Dried plasmas have been used experimentally to treat or mitigate EOT, both in vitro and in vivo.

Plasma inhibits vascular endothelial compromise, attenuates inflammation, restores the endothelial glycocalyx, and mitigates organ injury induced by hemorrhagic shock and trauma [81–90]. Freshly thawed FFP and S/D spray-dried plasma have similar protective effects on endothelial permeability and inflammation in vitro [91, 92]. In mice, S/D spray-dried plasma mitigated pulmonary vascular permeability, restored vascular junction integrity, corrected base deficit, and decreased pulmonary inflammation induced by hemorrhagic shock [93]. Similar results were observed using lyophilized plasma, both in vitro and in vivo [84, 85].

Maintenance of endothelial function by dried plasma is also suggested by results of large animal studies. In a swine model of polytrauma, hemorrhagic shock, and coagulopathy, lyophilized plasma and FFP were equivalent in correcting coagulopathy [94, 95]. In another study, 7-day survival was improved with spray-dried plasma compared to resuscitation with Hextend® and was equivalent to treatment with whole blood [96]. In a swine model of polytrauma, hemorrhagic shock, and traumatic brain injury, lyophilized plasma was effective in decreasing brain lesion size and improving long-term outcomes [97–99].

Taken together, the experimental data suggest that all currently available dried plasmas would be expected to perform similarly to FFP in the treatment of EOT. In preclinical studies, dried plasmas performed similarly to FFP regardless of whether the product was pooled or single donor, whether the method of drying was spray-drying or lyophilization, and whether or not the product had undergone S/D treatment for pathogen reduction.

An underlying concept in the use of plasma transfusion for the treatment or prevention of trauma-induced coagulopathy is that addition of plasma, with its normal balance and levels of pro-/anticoagulant and pro-/antifibrinolytic proteins, will mitigate plasma phase imbalances associated with the coagulopathic state (i.e., move them toward normal balance) [100–102]. In this regard, dried plasma products should be expected to exhibit an acceptable procoagulant to anticoagulant balance, similar to FFP. A number of clinical reports have demonstrated improved coagulation parameters (e.g., INR) following early transfusion of reconstituted dried plasma to patients with traumatic hemorrhage [52, 62, 68]. Nonetheless, it is informative to consider the profiles of in vitro hemostatic parameters of dried plasma products relative to FFP, as they may be impacted by methods of drying and pathogen reduction.

Lyophilization

Bux et al. examined changes in hemostatic parameters in LyoPlas N-w by comparing the starting FFP with the lyophilized product [42]. Lyophilization resulted in changes of generally <10% in hemostatic parameters. The most significant was vWF activity (~25% loss). All parameters remained in the normal range. Similar results were obtained in a study of FLYP [103]. Lyophilization of amotosalen-treated pooled plasma produced little change in coagulation factors, with lyophilization changing most factors by less than 10%. Exceptions included factor V (25% decline) and factor VIII:c (20% decline). All factors remained in the normal range except factor V. Thromboelastometric parameters were unchanged. Thrombin generation using high tissue factor reagent was unchanged, but was slightly reduced with low tissue factor [103]. In another study, concentrated LyoPlas N-w was added to reconstituted whole blood in vitro. Coagulation factor levels were increased above normal, but not thrombin generation, likely reflecting maintenance of normal ratios of procoagulant to anticoagulant proteins [104].

Freeze-drying appears to have little effect on proteins of the fibrinolytic system. Plasminogen levels remained above 90% [37, 42, 103] and alpha-2 antiplasmin levels remained above 80–90% [42, 103] after lyophilization. Addition of pooled lyophilized plasma to native whole blood in a thromboelastographic assay did not alter LY30 (clot lysis). However, addition of pooled lyophilized plasma to whole blood challenged with t-PA resulted in a significant reduction in the hyperfibrinolytic effect of t-PA, suggesting that, similar to FFP, dried plasma has an important "buffering effect" on the fibrinolytic system [105].

Spray-Drying

Liu et al. recently reported optimized processing procedures for a single-unit spray-dried plasma. The spray-dried plasma performed comparably to FFP in global assays such as PT, aPTT, and thromboelastography [41]. Factor levels were greater than 80% of FFP for all factors, except FXIII (74%) and vWF (60%). In vitro study of this spray-dried plasma found that, despite a reduction in high molecular weight vWF multimers, platelet adhesion was not impaired and overall clot characteristics remained within the normal range for FFP [106]. In a study of another spray-dried plasma, Spinella et al. reported that, despite reductions in individual factor levels during spray-drying, thrombin generation was greater with spray-dried plasma than with FFP and liquid plasma [48], raising the possibility of increased thrombotic issues. Data suggest that function of spray-dried plasma would be similar to lyophilized plasma, but a potential for thrombotic risk has been noted in vitro. No spray-dried plasma is yet commercially available. It will be important to consider the specific characteristics of any future product.

Pathogen Reduction

Both S/D and amotosalen pathogen reduction processes have been combined with drying procedures to produce pathogen-reduced dried plasma [37]. The processes have different effects on coagulation factor levels. Amotosalen treatment reduced factor VII (23% reduction), factor VIII:c (27% reduction), and fibrinogen (28% reduction), while other factors remained relatively unchanged [40]. The impact of amotosalen treatment on proteins of the fibrinolytic system appears small. Available data demonstrate that amotosalen treatment reduces plasminogen and alpha-2 antiplasmin levels by less than 10% [40]. FLYP incorporates amotosalen treatment for pathogen reduction [36]. There have been no adverse clinical reports related to altered factor levels for FLYP.

The largest reductions in factor levels following S/D treatment were for vWF activity (24% reduction), factor V (37% reduction), and protein S (44% reduction). Other factors were reduced to a lesser degree [40]. Proteomic changes have also been observed [107]. Solvent detergent treatment has little if any effect on plasminogen, but decreases alpha-2 antiplasmin activity to

approximately 20–40% of normal levels [39, 40, 107]. There has been some controversy surrounding S/D-treated plasma, specifically pertaining to the potential effects of reduced protein S and reduced alpha-2 antiplasmin. The extensive experience with liquid S/D plasma provides important information. Due to reduced levels of protein S and alpha-2 antiplasmin, the package insert for Octaplas liquid S/D plasma carries warnings about thrombosis risk (due to low protein S levels) and hyperfibrinolysis (due to low antiplasmin levels; Octaplas package insert). In vitro studies examining the impact of low alpha-2 antiplasmin and low protein S in liquid S/D plasma have demonstrated that t-PA-initiated whole blood clot lysis was more rapid than in FFP and that thrombin generation was enhanced. Addition of tranexamic acid mitigated the enhanced fibrinolysis [108–110]. Because both alpha-2 antiplasmin and protein S are synthesized by the liver, particular attention has been given to the use of liquid S/D plasma for patients undergoing liver transplantation.

A previously marketed liquid plasma product, Plas+SD (Vitex, Melville, NY), was removed from the market due to serious adverse events (thrombosis in liver transplant patients) that may have been associated with low protein S concentrations, elevated lipoprotein A, and complement activation in the product [39]. Current products incorporate a different S/D pathogen reduction process. An increased incidence of hyperfibrinolysis in a retrospective study of patients that received S/D plasma (Octaplas) during liver transplantation, as compared to patients that received FFP, has also been reported. Patients that received S/D plasma had increased plasma levels of D-dimer and fibrin degradation products. However, there was no difference in clinical outcomes or transfusion requirements [111]. Subsequently, a study of 195 consecutive primary liver transplant patients demonstrated low incidences of thromboembolism and hyperfibrinolysis in patients that received S/D plasma (Octaplas). The researchers concluded that the relatively low concentrations of α2-antiplasmin and protein S in Octaplas, compared to products that have not undergone pathogen reduction, such as FFP, do not seem to have a major clinical impact [112].

Bioplasma FDP, which is produced by lyophilization of S/D-treated plasma, has a contraindication for patients with severe protein S deficiency (Bioplasma package insert). Although there are no published data on factor levels in Bioplasma FDP, the package insert states that all factor levels are 0.4 IU (40%) or higher (Bioplasma package insert). Nonetheless, formal hemovigilance programs covering many hundreds of thousands of units of S/D-treated plasma, both liquid and lyophilized, have demonstrated an excellent record of safety [37, 113].

Taken together, it appears that, as long as clinicians are aware of the potential risks due to reduced alpha-2 antiplasmin and protein S in S/D plasmas, currently available dried plasma products may be used interchangeably with FFP for the treatment of trauma-induced coagulopathy.

Summary

Recent clinical trials have demonstrated that early plasma transfusion reduces mortality in patients with trauma and hemorrhagic shock. Prehospital administration of blood products, including plasma, has been adopted by many helicopter emergency medical services. However, logistical constraints still limit the use of plasma in helicopters and, to a greater degree, ground ambulances. A dried plasma product, with reduced cold-chain requirements and easy preparation, is needed to facilitate administration of plasma in the out-of-hospital environment. Three modern dried plasma products (Bioplasma FDP, FLYP, and LyoPlas N-w) are currently available in some countries, but dried plasma remains unavailable in most countries, including the United States. In addition to lyophilization, extra precautions, such as pathogen reduction, are used to ensure product safety. Additional products are in development. Experience with dried plasma products to date suggests that they are safe and effective and that they can be used interchangeably with FFP for prevention or treatment of trauma-induced coagulopathy, with some precautions based on individual product specifications.

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43 Synthetic Blood Substitutes

Anirban Sen Gupta

Introduction

Trauma-associated exsanguinating hemorrhage and trauma-induced coagulopathy (TIC) remain major causes of mortality, globally, both in the battlefield [1–5] and in civilian settings [6, 7]. In such severe hemorrhage scenarios, transfusion of whole blood (WB) or blood components (RBC/platelets/plasma in controlled ratios) can significantly improve hemostasis, mitigate hemorrhagic shock, and save lives. In fact, the US Army had documented the field use of group O whole blood transfusion to treat combat trauma during World War I and World War II [8–10]. The US military had also established strategic and logistic measures for "walking blood banks" in far forward austere battlefield settings [11–13]. With the development of blood fractionalization technologies, civilian blood banks adapted component-based therapy with the rationale for avoiding transfusion transmitted diseases. In recent years, a robust volume of clinical studies, including *PROMMTT, PROPPR, COMBAT*, and *PAMPer* trials, have established that mortality from severe hemorrhage and coagulopathy usually happens within hours post-injury, and early transfusion of WB or blood components, if available, can significantly improve survival [14–17]. Such clinical findings have led to the concept of damage control resuscitation (DCR) [18–20], including remote DCR (RDCR) which emphasizes the critical significance of making blood products accessible as close as possible to the hemorrhaging patient, at point of injury (POI), and en route, to reduce the time gap between injury and treatment and thus significantly improve survival. For example, the Committee on Tactical Combat Casualty Care (CoTCCC) has recently recommended WB for RDCR hemostatic resuscitation in combat trauma, and this has led to the development of a WB program by the US Army's 75th Ranger Regiment (Ranger Group O Low Titer or ROLO Program) to enable WB transfusion at POI [21–23]. Due to the blood collection challenges that can occur in the battlefield, the Armed Services Blood Program (ASBP) has begun collecting and shipping cold-stored low titer O WB to US military units in the field, and such WB is also currently in limited use in the civilian emergency services including prehospital transfusion in trauma, strongly endorsed by the AABB [24–26].

While such transfusion strategies are highly effective in saving lives, these approaches are obviously still heavily dependent on "donor blood." Also, these transfusion options are of very limited availability beyond large civilian and military trauma treatment facilities. This is mainly due to the challenges associated with por-

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tability, storage, contamination, and shelf-life of blood products. Firstly, there is a persistent global shortage of blood donors [27, 28], which affects availability. Additionally, fresh WB needs to be used within 24 h, cold-stored WB preferably within 10 days, and isolated RBCs within 20–40 days, and isolated platelets have a shelf-life of only ~5 days at room temperature, all of which pose severe logistical challenges regarding adequate availability and timely transfusion [29, 30]. Extensive efforts are currently being directed at reducing contamination and increasing the shelf-life of blood products. Prominent efforts in this area include development of pathogen reduction technologies (PRT) as well as storing blood products at reduced temperatures (cold-stored, cryopreserved, lyophilized, etc.) [31–34]. In parallel, important research efforts are currently being directed toward the in vitro production of donor-independent RBCs and platelets (a field of research interestingly termed "blood pharming") from progenitor cells and stem cells, utilizing unique bioreactor designs and culture conditions [35–40]. An alternative (and complimentary) approach to these strategies is the design of biosynthetic and artificial blood cells that functionally mimic the biological mechanisms of these cells while allowing large-scale in vitro manufacture, sterilization and storage for long time periods, universal applicability (no need for type matching), and widespread availability both within hospitals and prehospital. The current chapter will comprehensively review such synthetic blood substitutes.

RBC Substitutes and Oxygen Carrier Systems

In blood, the primary function of RBCs (Fig. 43.1a) is the transport of oxygen and partial transport of carbon dioxide to and from tissues, via binding of the gases to the protein hemoglobin (Hb) contained within the RBCs. The average

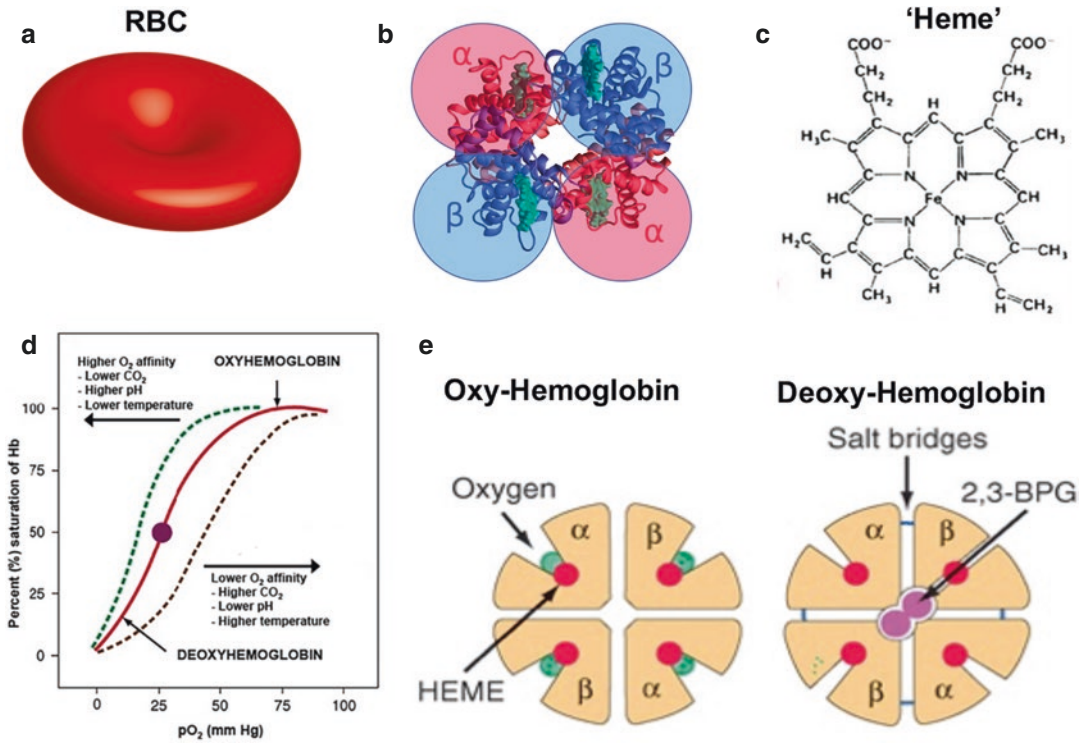


Fig. 43.1 Schematic representations of (**a**) RBC, (**b**) hemoglobin (Hb) within RBC, (**c**) "heme" metalloporphyrin molecule, (**d**) oxygen-binding curve of Hb, and (**e**) DPG-regulated oxygen loading and off-loading in Hb

amount of Hb in adult human RBCs is ~30 picograms per cell (~250 million Hb molecules). Hb is present as a tetrameric protein of two α- and two β-polypeptide units (Fig. 43.1b), each bearing an iron-containing "heme" group that binds one oxygen (O_2) molecule (Fig. 43.1c). O_2 binding to Hb is positively cooperative, such that a small variation in oxygen partial pressure (pO_2) causes a large change in bound O_2 by Hb, shown as a sigmoidal binding curve (Fig. 43.1d) [41]. The O_2-carrying iron in Hb is in its reduced "ferrous" (Fe^{2+}) state, which when oxidized to the "ferric" state (Fe^{3+}) is incapable of binding oxygen [41–44]. Therefore, in natural RBCs the O_2 transport mechanism of Hb is coupled to redox systems (e.g., driven by enzyme cytochrome b5 reductase), such that the Fe in Hb is consistently maintained in its O_2-binding state. Furthermore, Hb within RBCs undergoes conformational changes to allow saturation with O_2 in the lungs (higher O_2 affinity) and desaturation of O_2 (i.e., release) in the tissue capillaries (lower O_2 affinity). Such reversible conformational control of Hb to modulate O_2 affinity is mediated by allosteric effector molecules like 2,3-diphosphoglycerate (2,3-DPG) formed inside RBCs as a glycolytic intermediate (Fig. 43.1e). Therefore, engineering of a synthetic RBC substitute poses formidable challenges regarding incorporation of such thermodynamic and kinetic aspects of O_2 transport by Hb. The three main types of RBC-inspired oxygen carrier systems that have undergone extensive preclinical and some clinical research so far are Hb-based oxygen carriers (HBOCs), perfluorocarbon (PFC)-based emulsions, and iron (Fe^{2+})-containing porphyrin systems.

HBOC Systems

HBOC systems are "biosynthetic" mimics of RBC, since they utilize Hb from biological sources as the oxygen-carrying component. In HBOCs, Hb is used either directly in chemically modified forms or further conjugated to polymers for circulation stability or encapsulated within particle carriers [45–48]. The most common sources of Hb used in such technologies are outdated human RBCs, bovine RBCs, or from recombinant technologies [49–52]. For human or bovine Hb, the protein is isolated via cell lysis, purified, and sterilized (e.g., by low heat). Such cell-free Hb presents low antigenicity and is able to bind oxygen effectively. However, early studies of transfusing such cell-free Hb in patients showed extensive renal toxicity and cardiovascular complications [53–56]. Cell-free Hb has a very short circulation lifetime, as the Hb tetramer rapidly dissociates into dimeric and monomeric forms that can bind to plasma immunoglobulins, and undergoes rapid clearance into the spleen, liver, and kidneys, leading to organ toxicity. Cell-free Hb and its dissociated derivatives also extravasate into subendothelial region and rapidly sequester nitric oxide (NO), converting NO into nitrate (dioxygenation reaction) and oxy-Hb to Met-Hb [57]. Since NO is a natural vasodilator, its sequestration by cell-free Hb causes vasoconstrictive and cardiovascular complications. Additionally, the "heme" in cell-free Hb has inherent inflammatory and toxic effects toward vascular endothelium [58]. Also, the absence of 2,3-DPG in cell-free Hb results in the lack of conformational modulation of the O_2 affinity of Hb, leading to suboptimal tissue oxygenation. For these reasons, direct utilization of human or bovine cell-free Hb is now deemed unsuitable for transfusion applications. Instead of RBC-sourced Hb, research efforts have focused on the development of recombinant Hb (e.g., in *E. coli*) where specific mutations can allow reduced dissociation and NO-binding capacities. However, the correct combination of mutations that can lead to an ideal recombinant Hb molecule is still unresolved [59–61]. Therefore, the majority of HBOC research approaches have focused on chemical modification of Hb, including cross-linking, polymerization, and macromolecular surface modifications, or Hb encapsulation within particulate carriers, to improve stabilization, safety, and function in vivo.

HBOCs with Chemically Modified Hb

Cell-free Hb can be both intra- and intermolecularly cross-linked to stabilize the protein and reduce dissociation. For example, the α-subunits

can be cross-linked using acylation with diaspirin, which resulted in the human Hb-based HBOC product HemAssist (Baxter, USA) [47, 62–64]. This product showed circulation time of ~12 h compared to <6 h for non-cross-linked Hb, but clinical trials with this product showed a 72% increase in mortality, leading to trial discontinuation [64]. Similar cross-linking of the α-subunits of recombinant Hb was done using glycine, resulting in a product called Optro (Somatogen, USA), which also showed risks of cardiac arrest and mortality in clinical trials [65–67]. In another approach, polymerized Hb was created by intermolecular cross-linking using glutaraldehyde (e.g., the products Hemopure from Biopure, USA, and PolyHeme from Northfield Labs, USA) and o-raffinose (e.g., the product HemoLink from Hemosol, Canada) [68, 69]. In these approaches, a persistent issue is the lack of precise control over the polymerization extent, and rigorous purification steps become necessary to ensure product quality. PolyHeme did not show major vasoconstrictive cardiovascular effects in safety studies [67, 70] and progressed into phase III clinical trials in the USA in the 1990s in the Acute Normovolemic Hemodilution (ANH) trials, as well as in trials treating patients suffering from traumatic hemorrhagic shock under emergency research waiver (ERW) and special protocol assessment (SPA) framework implemented by the FDA. However, several fatalities occurred in such trials and the studies were abruptly shut down in 2001. Clinical trials with Hemopure (BioPure) showed favorable safety profiles and a reduced need of blood transfusions in cardiac surgery [71]. This product is approved in South Africa for acutely anemic patients, but is not yet approved by the FDA. The company developing the product is now known as HbO_2 Therapeutics, and the product (now known as HBOC-201) is currently undergoing further clinical evaluation. HemoLink also advanced to phase III clinical trials but was discontinued in 2003 when patients experienced adverse cardiac events. In fact, many of these products in their clinical trials have shown high risks of transient hypertension, microvascular constriction and dysfunction, gastrointestinal distress, nephrotoxicity, neurotoxicity, and increased mortality [72, 73].

Instead of intra- or intermolecular cross-linking, Hb has also been modified with macromolecular bioconjugation to increase circulation stability and reduce immune reaction [74–77]. This approach is demonstrated in polyethylene glycol (PEG) modification of Hb (e.g., the products Hemospan from Sangart Inc., USA, and PEG-Hb from Enzon, USA) and poly(oxyethylene) modification of pyridoxylated cross-linked Hb (e.g., the product PHP from Apex Bioscience, USA). PEGylated Hb products have undergone extensive clinical trials and the studies showed risks of bradycardia and elevation of hepatic pancreatic enzymes even at low doses [77]. Nonetheless, the phase I and phase II clinical trials with Hemospan showed favorable tolerance in humans for oxygen transport. However, subsequent phase III trials in orthopedic surgery patients in Europe showed continued risks of cardiovascular and renal dysfunctions [78]. The persistent issue of Hb being potent scavengers of NO via rapid irreversible binding has become a critical impediment toward clinical success of such products [79, 80]. Interesting solutions to this issue are the approaches of modifying Hb molecules to become NO carriers through S-nitrosylation of cysteine residues in the β-subunits of Hb or enzymatic transformation of Hb into becoming an NO donor in the presence of nitrites, but these approaches still need to be successfully established for in vivo efficacy [81]. Natural RBCs contain enzymes like catalase (CAT) and superoxide dismutase (SOD) that help mitigate the oxidative stresses stemming from superoxide moieties in injured and ischemic tissues. Based on this rationale, these enzymes have been cross-linked to polymerized Hb to form PolyHb-SOD-CAT, which showed combined advantages of long circulation time and reduced oxidative damage [82, 83]. Another approach looked at incorporating regulatory molecules such as 2,3-DPG and methemoglobin reductase along with Hb in HBOC systems, to prevent irreversible Hb oxidation and unfavorable O_2 affinity. In a recent product named HemoTech, purified bovine Hb was cross-linked intramolecularly with ATP and intermolecularly with adenosine and conjugated with reduced glu-

tathione (GSH) [84], with the rationale that ATP will regulate vascular tone through purinergic receptors, adenosine will counteract the vasoconstrictive properties of Hb, and GSH will shield the "heme" from NO and reactive oxygen species. The early-phase studies have shown promising properties of HemoTech. In a different approach, PEG conjugation was carried out on bovine carboxyhemoglobin (CO-Hb) instead of Hb, and the resultant PEG-CO-Hb system was evaluated for oxygen (and CO) transport capabilities [85–87]. The rationale behind this unique design is that, endogenous CO produced from (hypoxia-enhanced) heme-oxygenase activity can provide cytoprotective and vasoprotective effects. The PEG-CO-Hb product (Sanguinate, Prolong Pharmaceuticals, USA) has undergone preclinical evaluation and is currently in clinical trials for treating sickle cell anemia, thrombotic thrombocytopenic purpura (TTP), and ischemia.

In another recent approach, human serum albumin (HSA) was conjugated onto Hb by reacting Hb surface lysines to HSA cysteine-34 using α-succinimidyl-ε-maleimide cross-linker [88]. These core-shell Hb-HSA clusters are expected to have high circulation stability and can be further modified by incorporating antioxidant enzymes in the HSA pockets for protection of Hb [89]. These newer designs have only been evaluated in vitro, and rigorous preclinical in vivo studies would be needed to establish clinical promise. Figure 43.2 shows the above-described prominent HBOC designs based on chemically modified Hb.

HBOCs with Encapsulated Hb

In the pharmaceutical field, microparticle- and nanoparticle-based carriers are often used to encapsulate drugs and bioactive compounds to protect the payload from plasma effects, improve

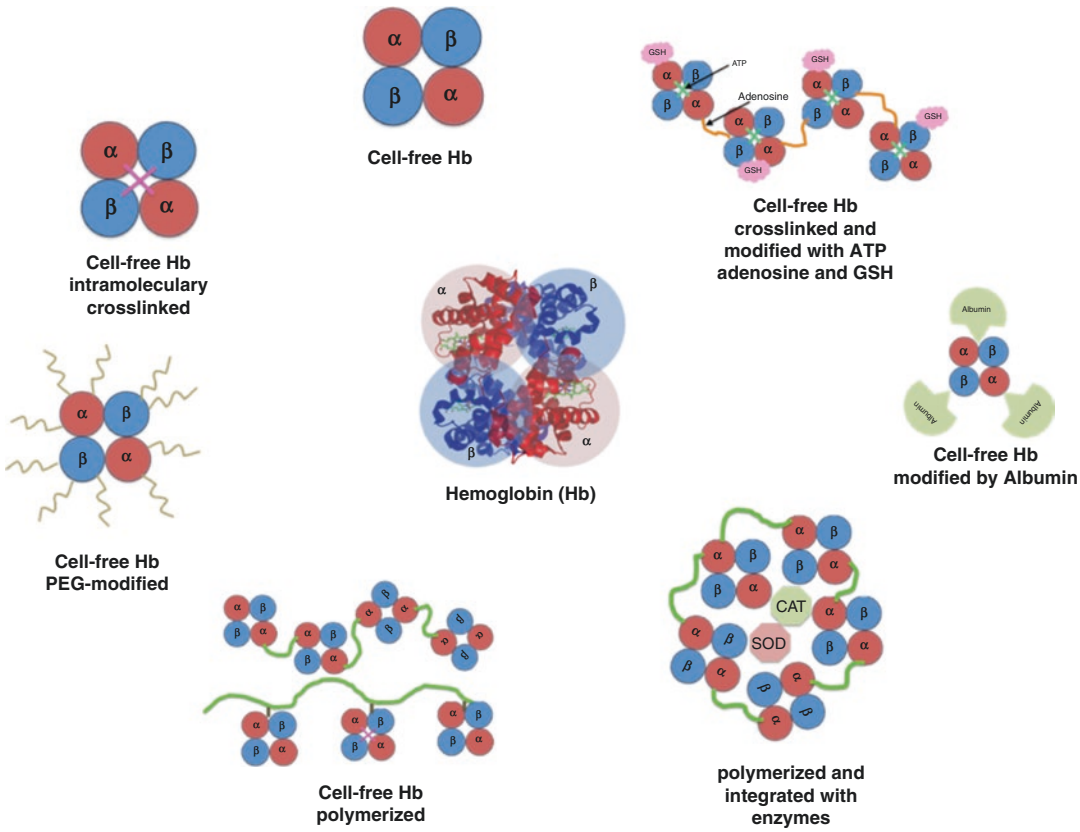


Fig. 43.2 Designs of Hb-based oxygen carriers (HBOCs) with cell-free Hb in chemically modified and polymerized forms

its circulation lifetime, and potentially allow improved delivery to target tissues. Similar approaches have been adapted to encapsulate Hb and create an "RBC substitute." The earliest demonstration of this approach is found in the pioneering work by Chang et al. during the 1950s, where Hb and enzymes were encapsulated within polymeric microcapsules. For this, the membrane material used was collodion (cellulose nitrate) or polyethylene glycol-polylactide (PEG-PLA) [90–92]. In vitro, these RBC-mimetic "hemoglobin corpuscles" showed oxygen binding curves similar to RBCs and retained activity of RBC-relevant co-encapsulated molecules like 2,3-DPG and CAT. However, a major challenge of using these systems in vivo is their rapid macrophagic clearance, resulting in very short circulation time. Reducing the particle diameter to ~1 micron partially improved the circulation profile, and further research efforts were directed at improving the circulation time by modifying the particle surface with lipids and polysaccharides. In another approach, Hb was encapsulated within micron and submicron sized lipid vesicles (termed liposome-encapsulated Hb or LEH), where the membrane contained phospholipids and cholesterol [93–95]. Several variations of this design have been studied, e.g., "Neohemocytes," "TRM-645 Neo Red Cells," etc., where the emphasis has been on maintaining uniform Hb encapsulation extent, consistent size distribution of the vesicles, and optimal vesicle stability in storage while conserving the oxygen transport properties of the encapsulated Hb [96, 97]. During the 1990s the "Stealth Liposome" technology was established, where liposomal nanoparticles (100–200 nm in diameter) were surface-functionalized with polyethylene glycol (PEG) to improve their storage stability, reduce rapid macrophagic uptake in vivo, and enhance their circulation residence time [98, 99]. Consequently, this technology was adapted to design Hb-encapsulated PEGylated liposome vesicles (HbV) [100–106]. In HbVs, 1,2-dioctad ecadienoyl-sn-glycero-3-phosphatidylcholine (DODPC) was used as a membrane phospholipid, such that γ-irradiation-based sterilization also produced hydroxy (OH) radicals that then promoted intermolecular cross-linking of dienoyl groups in DODPC to result in highly stable liposome membrane that could withstand severe temperature changes (e.g., lyophilization and rehydration). The HbVs have shown substantial improvement of circulation lifetime (~60 h in some animal models) and continue to show highly promising capabilities as a synthetic RBC substitute [105–107]. HbVs also reduce the NO-scavenging issues associated with cell-free Hb and also prevent clearance of Hb into the kidneys and other organs, thereby reducing organ toxicity risks. Newer designs of Hb-loaded liposomal systems have also been reported in recent years, termed liposome-encapsulated hemoglobin (LEH) [108, 109]. The HbV and LEH systems continue to be evaluated for potential clinical use as a synthetic RBC substitute in transfusion approaches in perioperative settings, massive hemorrhagic shock and hemodilution, oxygenation of ischemic tissues, and preservation of organ transplants.

Besides LEH and HbVs, encapsulation of Hb has also been studied in other particle systems, especially made of polymers like PEG-PLA, poly(ε-caprolactone)/poly(L-lactic acid) (PCL/PLA), poly(lactic-co-glycolic acid) (PLGA)/PEG copolymers, etc. [110–117]. Such amphiphilic block-copolymer systems form polymer vesicles, also known as polymersomes, analogous to liposomes. These polymersome systems can encapsulate Hb, leading to polymersome-encapsulated Hb (PEH) products. Hb loading in these PEH systems is usually at 1–2 mg/ml, which is much lower compared to RBCs (~150 mg/ml). These PEH systems can enable oxygen transport analogous to RBCs. While this suggests potential of the PEH systems toward application as a synthetic RBC substitute, extensive in vivo evaluation of these systems is currently lacking. HBOC designs have also been created by coprecipitating Hb with calcium carbonate ($CaCO_3$), followed by cross-linking with glutaraldehyde and selective dissolution of $CaCO_3$, to form Hb-encapsulated microparticles [118]. Analogous Hb microparticles carrying about 80% Hb content compared to natural RBCs have also been reported where Hb and manga-

nese carbonate ($MnCO_3$) were coprecipitated, followed by human serum albumin-mediated encapsulation and stabilization [119]. These particles have reduced NO scavenging and related effects on vasoconstriction. Some studies have also directly conjugated Hb to the hydrophobic or hydrophilic domain of block copolymers and subjected to self-assembly to form Hb-loaded micelles [120, 121]. In another recent approach, $MnCO_3$ nanoparticles were used as templates for layer-by-layer (L-B-L) deposition assemblies of Hb and dialdehyde heparin (DHP), followed by cross-linking to stabilize the layers and selective dissolution of the $MnCO_3$ core [122]. A similar approach was also used with layers of Hb, DHP, and the enzyme catalase (CAT), to create L-B-L assembled Hb-loaded nanotube systems [123]. These complex Hb-encapsulated structures have been characterized in vitro for their morphology, stability, cytotoxicity, and RBC-mimetic O_2-binding capacity, but no in vivo evaluation has been reported yet. In a more recent approach, polyethyleneimine (PEI) was modified with palmitic acid to create amphiphilic polymer conjugate and this was used to form toroidal shaped nanoparticles (termed nanobialys, ~200 nm diameter) that can encapsulate Hb, as well as maintain redox enzymatic environment for Hb activity by co-encapsulation of 2,3-DPG and leukomethylene blue [124]. These unique HBOC particles, termed Erythromer, have shown promising oxygen transport properties in vitro and in vivo, and detailed in vivo studies are currently ongoing regarding biocompatibility, circulation lifetime, stability, and hemorrhagic shock mitigation in trauma, to establish their potential as a synthetic RBC substitute. Interestingly, instead of encapsulating Hb, some approaches have also attempted to encapsulate O_2 directly within phospholipid microvesicles (2–4 μ in diameter) to be delivered to deoxygenated RBCs in circulation [125, 126]. Although these O_2-loaded microbubbles were found to be stable for a few weeks in storage with minimal oxygen loss, in vivo they showed a very short circulation lifetime (<1 h).

Some Hb encapsulation approaches have also focused on the biophysical properties of natural RBCs that significantly influence their oxygen transport functions. Natural healthy RBCs have biconcave discoid morphology, ~8 μm in diameter, and ~ 2 μm thickness and are highly flexible (Young's modulus 0.1–0.2 kPa), which allows efficient passage through microvascular circulation for oxygen transport [127–129]. These natural cues have recently led to mimicry of RBC size, shape, and flexibility parameters into Hb-encapsulating particles. For example, polyelectrolyte-driven L-B-L assembly systems have been created that mimic the shape and deformability of natural RBCs [130]. In one such design, Hb and BSA were electrostatically deposited on the surface of RBC-shaped PLGA particles of ~7 μm diameter and 400 nm shell thickness, and then the PLGA core was selectively dissolved to yield RBC shape-mimicking Hb-loaded deformable particles. Similar RBC-mimetic particles have been fabricated using PEG-based hydrogels in a stop-flow lithography (SFL) approach where the mechanical properties of resultant particles could be controlled by modulating cross-linking density of the hydrogel [131]. In another approach, RBC-mimetic particles were manufactured from acrylate hydrogels using a particle replication in nonwetting templates' (PRINT®) technology mold [132]. These unique RBC-mimetic Hb-loaded particles demonstrated elastic deformation capabilities when circulating through narrow channels in vitro, but their in vivo oxygen transport capabilities and transfusion medicine potential are yet to be evaluated in detail. In a different approach, liposome-encapsulated actin-hemoglobin (LEAcHb) synthetic RBCs with natural RBC-mimetic shape were manufactured using a polymerized actin core [133]. Although these particles were much smaller (~140 nm diameter) than RBCs, the biconcave shape along with the flexibility of the membrane improved the half-life significantly. In natural RBCs, the negative surface charge electrostatically prevents RBC aggregation over a distance of 20 nm, and this rationale has led to the mimicry of RBC-relevant surface charge on Hb-encapsulating PEG-PLA nanoparticles (<200 nm in diameter) using cetyltrimethylammonium bromide (CTAB) or anionic sodium dodecyl sulfate (SDS) surfactants [134].

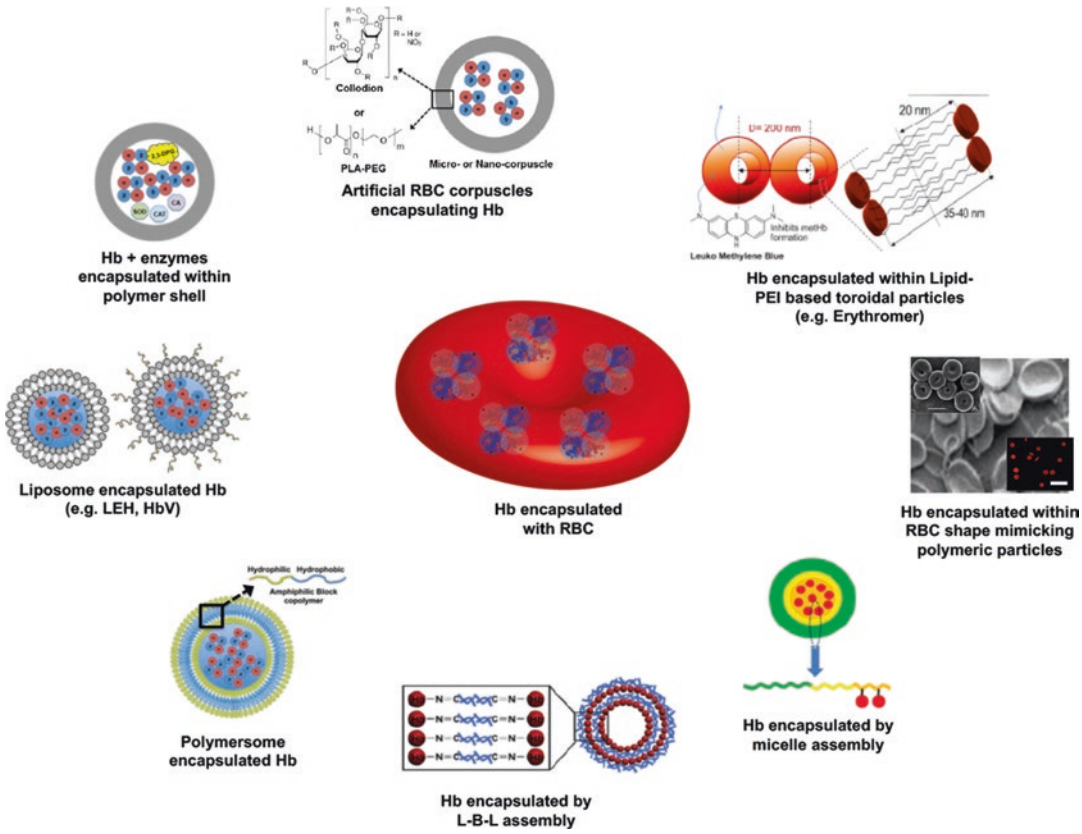


Fig. 43.3 Designs of Hb-based oxygen carriers (HBOCs) with Hb encapsulated within various nanoparticles and microparticles

Interestingly, cationized particles were found to have a half-life of ~11 h (eightfold higher than untreated particles), while the anionized particles were quickly eliminated, giving a half-life of <1 h. Figure 43.3 shows representative designs of HBOCs with encapsulated Hb systems that are currently undergoing in vitro and in vivo evaluation for application as a biosynthetic RBC substitute.

Perfluorocarbon (PFC)-Based Oxygen Carrier Systems

Due to the various challenges associated with HBOCs, significant research efforts have also been directed toward the development of non-Hb-based oxygen carrier systems. To this end, the most studied technologies are made of perfluorocarbons (PFCs). PFCs are biologically inert, and liquid PFCs have been extensively utilized as contrast agents in ultrasound imaging applications [135–138]. O_2 can loosely bind to PFC monomers by van der Waals-type interaction. This binding equilibrium of O_2 in PFCs follows Henry's law, resulting in a linear oxygen-binding profile, in contrast to the sigmoid binding curve of oxygen to Hb [139, 140]. Figure 43.4 shows chemical structures of some representative PFCs that have been investigated for oxygen transport applications, as well as the linear profile of O_2 binding to PFCs compared to the sigmoid profile of O_2 binding to Hb as well as representative HBOCs. The linear O_2-binding curve of PFCs implies that a much higher oxygen partial pressure is required to saturate PFCs with oxygen. On the other hand, due to the linear profile, oxygen desaturation (i.e., release) capacity is

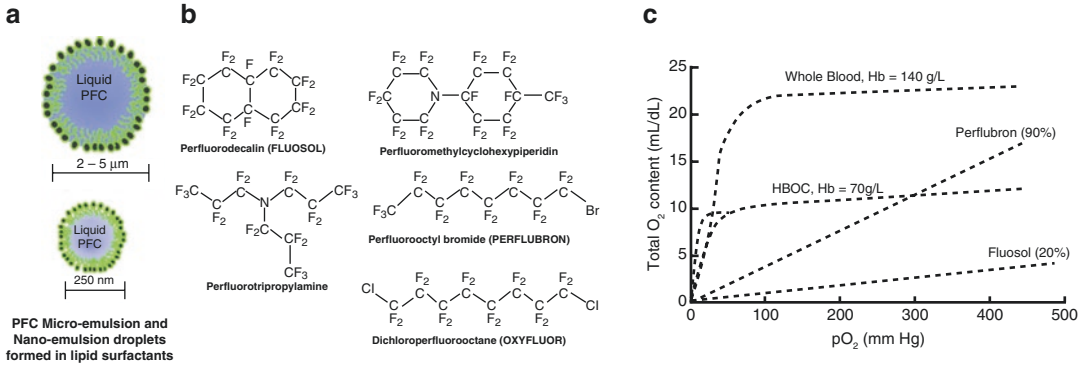


Fig. 43.4 (**a**) Perfluorocarbon (PFC)-based oxygen carrier designs where PFCs were stabilized by lipid-based nanoemulsions and microemulsions; (**b**) representative chemical structures of PFCs that have undergone significant research as oxygen carriers; (**c**) oxygen-binding curves of PFCs compared with HBOCs and whole blood showing that PFCs have linear O_2 saturation profile while HBOCs (and RBCs within whole blood) have sigmoidal O_2 saturation profile

better for PFCs compared to Hb. Due to immiscibility of PFCs in water, their in vivo application requires rigorous emulsification with suitable surfactants (e.g., phospholipids), and the oxygen solubility in PFC emulsions is independent of the surfactant used [141]. Several PFC emulsions have been investigated extensively as oxygen carriers. Fluosol-DA (Green Cross Corp., Japan), an emulsion of 10–20% perfluorodecalin in albumin, was extensively tested in animal models and progressed into clinical trials to receive FDA approval in 1989 [142]. However, this product was soon recalled because of issues like low oxygen transport capacity (0.4 mL O_2/100 mL compared to blood's reported O_2 transport capacity of 20.1 mL/100 mL), premature oxygen release, short shelf-life, temperature instability, slow excretion, and serious side effects. Fluosol also caused low platelet counts (thrombocytopenia), febrile symptoms, and macrophage activation (inflammatory trigger). These issues with Fluosol were somewhat resolved by newer-generation PFC compounds like perfluorooctyl bromide (Perflubron), perfluorodecyl bromide, and perfluorodichlorooctane [143–149]. These compounds in specific ratios were used along with egg yolk phospholipids, to create a PFC-based oxygen carrier Oxygent (Alliance Pharmaceutical Corp, USA), which showed reduced macrophage activation [146, 147]. Circulation half-life for Oxygent droplets was ~10 h for a dose of 1.8 g/kg in humans [149, 150]. Oxygent was found to render higher oxygen delivery compared to Fluosol, but was still ~30% less efficient than natural RBCs. Another analogous PFC product was developed using perfluorodicholorooctane, egg yolk phospholipid, and triglyceride, named Oxyfluor (HemaGen, USA), with droplet diameters in the range of ~250 nm [148], and showed properties similar to Oxygent. One advantage of these nanoscale PFC droplets is their ability to occupy small plasma volumes and efficient circulation through microcapillaries for oxygen delivery. In preclinical models, both Oxygent and Oxyfluor have shown promising benefit in oxygen delivery, although both products have also shown thrombocytopenic side effects [151]. In several large animal (e.g., canine) models of hemodilution and cardiopulmonary bypass, these PFC systems have shown promising ability to improve tissue oxygenation [152]. In fatal hemorrhagic shock models, these PFC systems improved resuscitation and reduced mortality significantly. These promising results have led to clinical evaluation of such products in human patients undergoing major noncardiac surgery, hemodilution, and hemorrhage [153–155]. Oxygent was evaluated as a substitute for autologous blood transfusion and was able to significantly transport oxygen and reduce transfusion

needs. These results indicated that such PFC-based oxygen carrier systems have the potential to act as RBC substitutes. However, advanced clinical trials of such products in patients undergoing cardiopulmonary bypass were terminated in the USA because of increased risk of stroke and adverse events, although deeper analysis of the data suggests that these adverse events may have been associated more with the study protocol rather than the PFC product itself [150]. Oxycyte (Oxygen Biotherapeutics Inc, USA), a third-generation PFC emulsion containing an aqueous 60% emulsion of perfluoro tert-butylcyclohexane with purified egg yolk phospholipids, has undergone evaluation in several animal models and some clinical trials in patients with traumatic brain and spinal cord injury [156–158]. Although some safety concerns were raised regarding Oxycyte's effect on the immune system and possible hemorrhagic side effects, recent animal studies have alleviated FDA concerns. Perftoran (Perftoran, Russia) is another PFC emulsion of perfluorodecalin and perfluoromethylcyclopiperidine in a nonionic surfactant that has been widely investigated in Russia [159, 160]. Although this product has shown adverse effects like hypotension and pulmonary complications, randomized clinical trials with Perftoran conducted in Mexico City on patients undergoing cardiac valvuloplasty showed higher intraoperative pO_2 levels and reduced need for allogenic RBCs. Further clinical studies are required to establish the transfusion benefit of such PFC products as RBC substitutes.

Synthetic Porphyrin Systems

The oxygen-transporting "heme" site of natural hemoglobin consists of a porphyrin tetrapyrrole ring surrounding an iron atom (Fig. 43.1c). Based on this, some researchers have studied Fe(II)-bearing porphyrin systems for oxygen transport [161–163]. For example, "picket fence" Fe^{2+} porphyrin molecules were developed that demonstrated reversible oxygenation of myoglobin and hemoglobin via immobilization of ferrous "heme" in a sterically hindered hydrophobic matrix [161]. These systems showed cooperative oxygen binding similar to natural Hb, but were prone to irreversible oxidation in aqueous media. To address this, a hydrophobic environment was created for these molecules, for example, using liposomes, where amphiphilic Fe^{2+} porphyrin bearing four alkylphosphocholine groups were embedded into the phospholipid bilayer (e.g., Lipidheme) [164, 165]. The resultant vesicles demonstrated reversible binding and release of oxygen similar to natural Hb. Instead of liposomes, HSA particles have also been used to incorporate these synthetic Fe^{2+} porphyrin systems, and these have shown RBC-mimetic oxygen-transporting efficiency. Surface modification of these particles with PEG has resulted in increased circulation time and reduced oxidation. Another porphyrin-based design is HemoCD, which is comprised of a 1:1 complex of 5,10,15,20-tetrakis (4-sulfonatophenyl) porphinato iron(II) (Fe[II] TPPS) and a per-*O*-methylated β-cyclodextrin dimer having a pyridine linker (termed HemoCD or Py3CD) [162]. This system has shown oxygen affinities similar to natural Hb and was found to be gradually autoxidized in aqueous environment. The circulation stability of these systems could be further enhanced by surface decoration with PEG-based dendrimers [166]. Although these various porphyrin-based oxygen carrier designs continue to be studied in vitro and in preclinical models [167], currently they lag behind HBOC and PFC systems in terms of translational advancement, possibly due to the challenges of large-scale manufacture and evaluation of these systems.

Platelet Substitutes

In blood, the primary role of platelets is to render blood clotting and hemostasis. Platelets are anucleated cells produced from mature megakaryocytes, and they provide constant surveillance of the blood vessel wall to rapidly enable hemostatic response in the event of an injury. Platelet's hemostatic response mechanisms at a bleeding site is multifold (Fig. 43.5): (i) rapid platelet

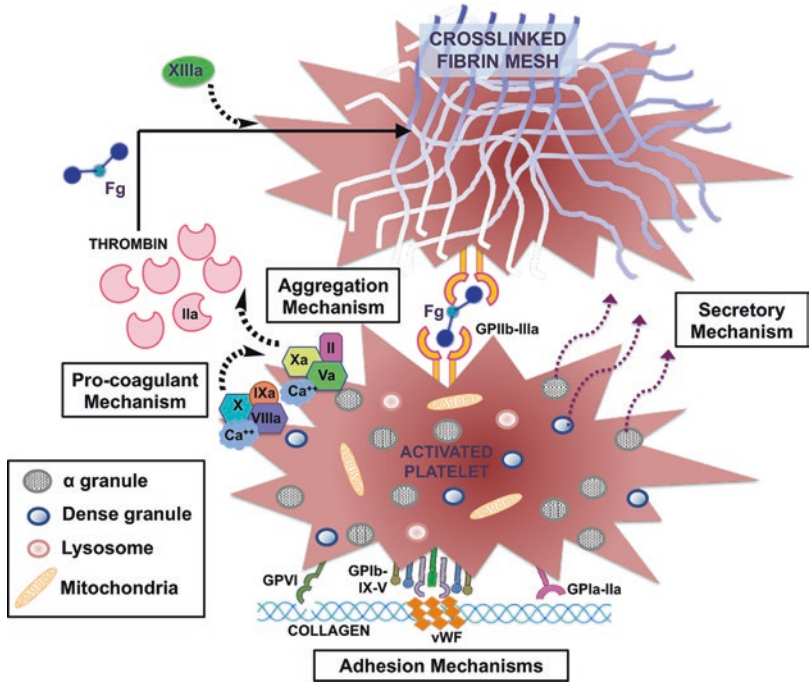


Fig. 43.5 Platelet-mediated mechanisms of hemostasis where platelets adhere to the injury site via binding to vWF and collagen, activated platelets aggregate at this injury site via fibrinogen-mediated clustering, the surface of these activated platelets amplify procoagulant mechanisms of thrombin generation and fibrin formation, and activated platelets also secrete a variety of granule contents that further enhance activation of localized platelet and coagulation factors, as well as fibrin morphology and stability to augment hemostasis

adhesion at the site by binding to specific proteins like von Willebrand factor (vWF) and collagen that are exposed at the site, (ii) adhesion-induced as well as agonist-induced platelet activation and rapid aggregation of activated platelets at the bleeding site via interplatelet bridging by blood protein fibrinogen (Fg) interacting with platelet surface integrin GPIIb-IIIa (primary hemostasis), (iii) amplification of procoagulant mechanisms (e.g., thrombin amplification and hence fibrin formation from Fg) via co-localization of coagulation factors on the negatively charged phosphatidylserine (PS)-rich membrane surface of active platelets, and (iv) enhancement of coagulation kinetics and clot stability/morphology via secretion of platelet granule contents like ADP, vWF and inorganic polyphosphate (PolyP) [168–174]. Hence, loss of platelets due to traumatic hemorrhage or surgery, as well as drug-induced or congenital defects in platelet number and function, can lead to severe bleeding complications and coagulopathy. As a result, transfusion of platelets is routinely used in the clinical mitigation of bleeding risks (prophylactic transfusion) and hemorrhage (therapeutic transfusion) of such conditions [31, 175, 176]. These transfusions primarily use allogeneic platelet suspensions stored at room temperature (20–24 °C) with gentle agitation, as per recent FDA guideline (FDA-2014-D-1814). However, in these storage conditions, platelets have a high risk of pathogenic contamination, resulting in very short shelf-life (~ 5–7 days) [31, 176]. This poses severe logistical barriers in widespread use of platelets within hospitals (unless they are large trauma centers with sufficient platelet availability), as well as prehospital use of platelet transfusions for point of injury and en route hemorrhage mitigation in civilian and battlefield scenarios. Significant efforts are currently being directed toward enhancing the storage and availability of platelets, via utiliza-

tion of pathogen reduction technologies, as well as reduced temperature storage (e.g., platelets chilled to 4 °C) and freeze-drying (e.g., Thrombosomes, manufactured by Cellphire, USA), and these are improving platelet storage and usage [32–34, 177–187]. However, these strategies and logistics are still dependent upon "donor platelet" availability, which remains a persistent challenge due to limited number of donors. Therefore, a significant parallel interest has emerged in creating "synthetic platelet substitutes" that can render efficient hemostasis while allowing advantages of large-scale manufacture, minimum contamination risk via effective sterilization, much longer shelf-life, and no need for blood type matching [188–192]. Based on the hemostatic response mechanisms of platelets, the "synthetic platelet" approaches have primarily focused on mimicking these mechanisms on biosynthetic and synthetic particle platforms. Figure 43.6 shows various representative designs of "synthetic platelet" systems.

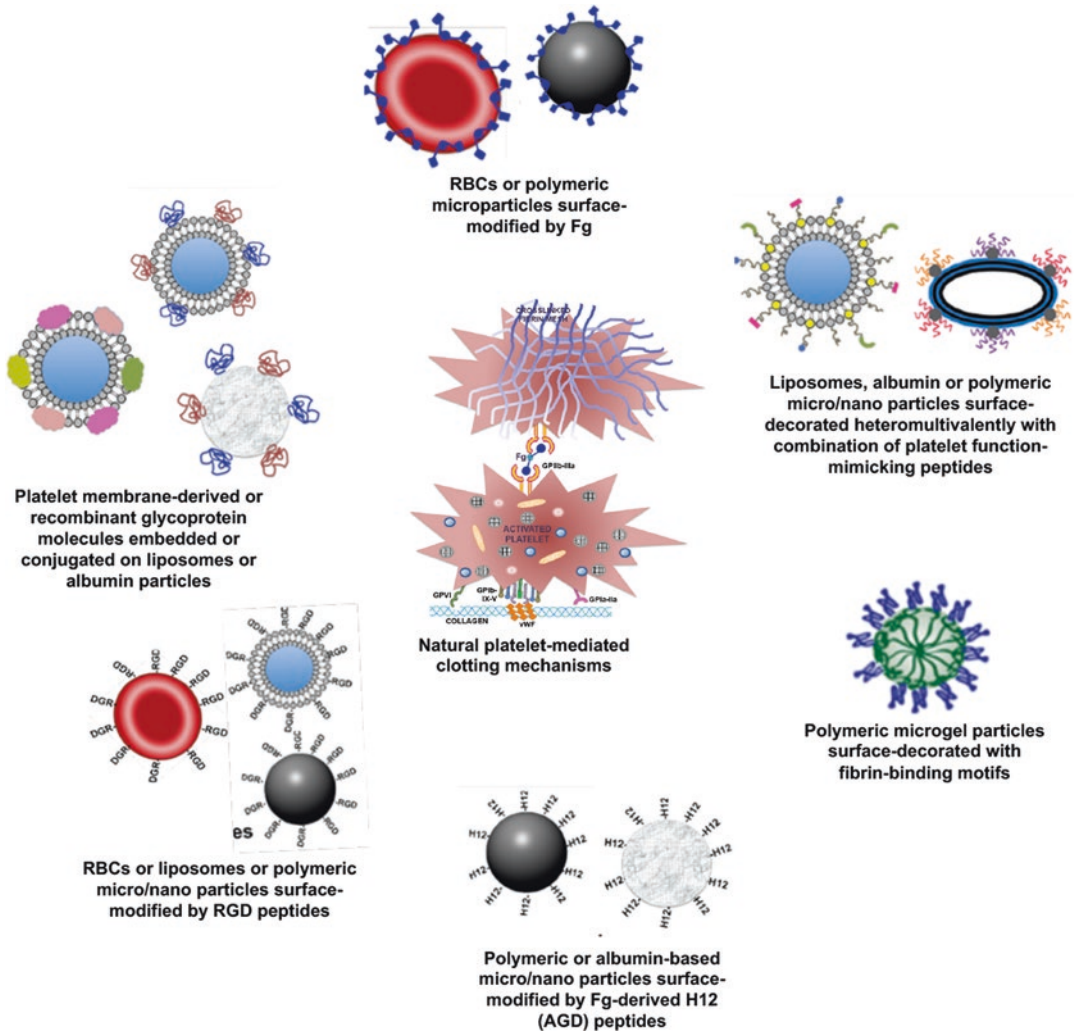


Fig. 43.6 Designs of "synthetic platelet substitutes" that mimic adhesive, aggregatory, procoagulant, and biomechanical aspects of platelet-mediated hemostatic mechanisms

Synthetic Platelet Designs Mimicking Platelet Adhesion Mechanisms

Adhesion of platelets to the bleeding injury site occurs through several specific mechanisms. The injured endothelial cells lining the luminal wall of blood vessels secrete von Willebrand factor (vWF) molecules from their Weibel-Palade bodies [193]. The injury site also exposes subendothelial collagen due to endothelial shedding/denudation. The vWF molecule is secreted as a globular protein, which unravels under hemodynamic shear and multimerizes to be deposited on injured endothelium and exposed collagen [194]. Platelets can bind to the A1 domain of this unraveled vWF via platelet surface receptor component GPIbα of the GPIb-IX-V complex [194, 195]. Concomitantly, platelet surface glycoproteins GPIa-IIa and GPVI can anchor onto the exposed collagen at the injury site, and this synergistically strengthens the platelet adhesion [195]. These adhesion mechanisms have inspired design of "synthetic platelet" constructs that emulate platelet's vWF-binding and collagen-binding capabilities. One such early design was "Plateletosomes," which involved detergent-based extraction of platelet membrane glycoproteins and their subsequent incorporation within the lipid membrane of liposomes [196]. While this approach allows retention of platelet's own hemostatically functional surface proteins, the extraction, purification, and liposome membrane incorporation steps make this strategy potentially too complicated to scale up and maintain batch-to-batch quality. An analogous approach was adapted by utilizing recombinant GPIbα (rGPIbα that binds to vWF's A1 domain) and GPIa-IIa (rGPIa-IIa that binds to collagen) to be conjugated on the surface of liposomes, latex beads, or albumin-based particles [197, 198]. In further advancement of this approach, both rGPIbα and rGPIa-IIa have been co-conjugated on the surface of liposomes and albumin particles, and this combination demonstrated higher binding to collagen surfaces in presence of soluble vWF at higher shear rates, closely mimicking natural platelet adhesion [199]. Recombinant technology can be quite expensive for clinical translation. Also, the large size of the recombinant protein fragments can lead to issues of mutual steric interference regarding their combinatorial decoration on the surface of nano- and microparticles. These issues can be potentially addressed by using small peptides that have vWF-binding and collagen-binding capabilities. An early example of this is found in reports of GPIbα-relevant 15-mer peptides that have binding capability to vWF [200]. These peptides were conjugated onto liposomal surfaces to create "synthetic platelet" constructs [201]. Although the biochemical characterizations of these constructs have been reported, reports of actual hemostatic efficacy evaluation of these constructs are not available. In a more recent approach, researchers have utilized a vWF-binding peptide (VBP) sequence TRYLRIHPQSWVHQI derived from the C2 domain (residues 2303–2332) of the coagulation factor FVIII and a collagen-binding peptide (CBP), which is a 7-mer repeat of the glycine(G)-proline(P)-hydroxyproline(O) tripeptide (i.e., -[GPO]7-) with helicogenic affinity to fibrillar collagen but minimal affinity to platelet collagen receptors GPIa/IIa and GPVI, to mimic platelet adhesion mechanisms on nanoparticles [202, 203]. VBPs and CBPs were conjugated onto liposome surface, and the resultant constructs showed significant platelet-mimetic adhesion on "vWF + collagen" surfaces in microfluidic channels at low-to-high shear ranges. This approach emphasizes the utilization of small peptides for hetero-multivalent functionalization of nanoparticles for synergistic bioactivity, e.g., in this case mimicry of the injury site-specific dual adhesion mechanisms of platelets.

Synthetic Platelet Designs Mimicking Platelet Aggregation Mechanisms

For forming the primary hemostatic plug, activated platelets aggregate via Fg-mediated bridging of the stimulated conformation of integrin

GPIIb-IIIa on platelet surface [204–207]. Integrin GPIIb-IIIa on activated platelets binds Fg via interaction with RGD and HHLGGAKQAGDV (also known as H-12) peptide sequences present in the α and γ chains on both termini of Fg [206, 207]. Based on this aggregation mechanism, several "synthetic platelet" approaches have attempted to mimic this by coating synthetic particle surfaces with Fg itself, Fg fragments, or Fg-based RGD and H-12 peptides. Earliest designs using this approach involved surface decoration of latex beads and RBCs with Fg or Fg-derived RGD peptides [208–211]. These designs are essentially "super-fibrinogen" systems where the particle (or RBC) surface decoration with Fg or Fg-derived RGD peptides is meant to amplify the aggregation of active platelets via multivalent interactions with GPIIb-IIIa integrins. These designs demonstrated hemostatic promise in vivo in several preclinical models, but have failed to progress into clinical translation. Another Fg-based "synthetic platelet" design that has undergone preclinical and clinical evaluation in the USA and Europe is Fibrocaps™ (ProFibrix, the Netherlands), where a solution of fibrinogen and thrombin is separately spray-dried and then combined to produce a mixture of suspendable microparticles [215]. Fibrocaps has been used in clinical trials for treatment of bleeding during surgery and trauma-related injury and has shown significant reduction in time to hemostasis after direct administration to surgical wound within a Gelatin Sponge, compared to the sponge alone. One limitation of this product is that it needs to be directly applied to the wound (sprayed or applied in a sponge) and therefore can only be applied to accessible wound sites and not intravenously. Several other Fg-based products that have undergone research as platelet-mimicking hemostatic technologies are Synthocytes™, Thrombospheres™, and Fibrinoplate™, all of which are essentially made of human albumin microparticles surface-coated with Fg [212–214]. These products have undergone preclinical in vivo studies and some early-phase clinical trials, but more detailed evaluations are needed to establish their clinical promise. Another interesting "synthetic platelet" design recently reported is fibrin-targeted micro-/nanogel systems that can respond to an injury site to stanch bleeding. This approach has been demonstrated with polyisopropyl acrylamide-based low-cross-linked mircogel particles surface-decorated with fibrin-specific recognition motifs [213]. Mechanistically, the binding of these constructs would require prior presence of sufficient fibrin (i.e., sufficient propagation of coagulation mechanisms) at the injury site, to augment hemostatic output. Instead of coating particles with Fg, resent designs have also utilized Fg-relevant small molecular weight peptides for particle surface decoration. Most of these approaches have utilized linear small RGD peptides, e.g., CGRGD or GRGDS, that have binding capability to platelet surface integrin GPIIb-IIIa [216, 217]. Two potential issues with using these peptides are (i) their ubiquitous nature to bind to many different integrins on other cells (i.e., lack of platelet specificity) and (ii) their reported ability to trigger activation of resting platelets (i.e., systemic prothrombotic risk) [218–220]. Nonetheless, from a feasibility standpoint, decoration of micro- and nanoparticles with these RGD peptides has resulted in platelet-mimetic constructs with promising hemostatic ability in vitro and in vivo [216, 217, 221]. The issue of platelet specificity can be potentially resolved by utilizing peptides that have higher selectivity to the active platelet GPIIb-IIIa. To this end, researchers have used Fg γ-chain relevant H-12 peptide or Fg function-mimicking GPIIb-IIIa-specific cyclic RGD (cRGD) peptides (e.g., cyclo-CNPRGDY[-OEt]RC) to decorate albumin, polymeric, or liposomal particles to create synthetic platelet mimics, with higher functional specificity in hemostatic action [222–225]. The H-12 peptide decorated liposomal particles have been further studied for targeted delivery of ADP (a platelet agonist) [226], while the cRGD peptide-decorated liposomal particles have been further studied for targeted delivery of tranexamic acid (TXA, an antifibrinolytic agent) [227], to augment hemostatic capability. All of these "synthetic platelet" designs mimicking the aggregation mechanism of platelets have shown hemostatic promise

in vivo in a variety of preclinical animal models including tail bleeding, femoral artery bleeding, liver laceration, and blunt trauma. Clinical translation of these designs will require establishing efficient large-scale manufacturing processes, demonstrating sterilizability and long shelf-life without compromising bioactivity, and evaluating systemic safety, circulation lifetime, and hemostatic efficacy in large animal models.

Synthetic Platelet Designs that Combine Platelet's Adhesion and Aggregation Mechanisms

Platelet-mediated hemostasis is rendered by the cooperative action of the adhesive and aggregatory functionalities of platelets [168–170, 228]. Therefore, in recent years there has been growing interest in developing "synthetic platelet" designs that combine these two mechanisms on a single particle platform. To this end, latex beads were surface-decorated simultaneously with rGPIbα protein fragments, and H-12 peptides and liposomes were surface-decorated with rGPIbα protein fragments and collagen-binding peptides [229]. Studies with these approaches revealed that in combining two different motifs on a nanoparticle surface if there is a significant size difference between the motifs (e.g., large rGPIbα fragment compared to small H-12 peptides), the motifs need to be presented at different canopy levels from the particle surface by using different spacer lengths, in order to minimize mutual steric interference. Such issues can be potentially resolved by using only small peptides to combine the adhesive and aggregatory functions of platelets. This was demonstrated by combining the adhesion-promoting peptides VBP and CBP with the aggregation-promoting cyclic RGD-based Fg-mimetic peptide (FMP) on liposomes or albumin-based particles [225, 230–232]. These "functionally integrated" platelet-mimetic systems could be scaled up, sterilized, and stored over 6–9 months period of time, without compromising hemostatic bioactivity [232]. These "synthetic platelet" systems showed higher hemostatic efficacy in vitro, as well as in vivo, compared to systems that had *adhesion only* or *aggregation only* mechanism. The liposome-based functionally integrated "synthetic platelet" design was recently issued multiple patents and registered as "SynthoPlate." This technology is currently being tested in large animal models of traumatic hemorrhage, to assess clinical promise. The "heteromultivalent surface decoration" to combine platelet's adhesive and aggregatory function also remains amenable to be adapted to other biomedical particle systems, as was demonstrated by combining VBP, CBP, and FMP decorations on albumin-based platelet-shaped (discoid) nanoparticles that exhibit superior wall margination capabilities [233]. These studies suggest that mimicking platelet-relevant multiple hemostatic mechanisms via combinatorial ligand modifications on nanoparticles and microparticles may lead to unique and improved synthetic platelet designs.

Discussion

Synthetic blood substitutes remain highly desirable in transfusion medicine to potentially address the persistent logistical and functional challenges associated with the widespread use of natural blood products. The use of milk, saline, Ringer's solution, or animal-derived plasma to substitute for human blood has been recorded in history during the nineteenth century [234]. These materials could partly act as volume replacement systems but obviously failed to render the oxygen transport and hemostatic functions of the natural blood cells. Volume replacement approaches have also led to the subsequent development of "plasma expanders," namely, crystalloids (e.g., sucrose, dextrose, etc.) and colloids (e.g., albumin, hydroxyethyl starch, etc.) [235, 236]. However, in massively hemorrhaging patients, excessive use of such fluids has actually led to further deleterious effects, aggravating shock and dilutional coagulopathy [237, 238], leading to clinical guidance of minimizing such excessive use of fluid-based resuscitation (e.g., permissive hypotension). Through robust clinical studies during the recent decade, we have

now come full circle to establish that human whole blood (WB), if available, is the best transfusion product to mitigate hemorrhage and coagulopathy. If WB is not available, the next best choice is transfusion of isolated blood components (platelets, RBC, plasma) at controlled ratios. However, the availability of WB or components is still limited by donor availability, special storage requirements, rigorous hemovigilance and blood banking, and lack of widespread portability beyond major hospitals and large trauma centers. This is where "synthetic blood substitutes" are envisioned to provide a potential "bridge" solution, by allowing large-scale manufacture, sterilization and long-term storage as suspension or aqueous-reconstitutable freeze-dried powder, and on-demand rapid transfusion both within hospitals and in prehospital settings. Newer transfusion logistics can also be developed by mixing or co-formulating "RBC surrogates" and "platelet surrogates" in plasma to constitute a "whole blood surrogate," and the promise of such approaches was recently demonstrated by combining H-12 peptide-decorated ADP-loaded liposomal particles with HbVs in transfusion management of massive hemorrhage in thrombocytopenic rabbit models [239]. Synthetic blood substitutes can also allow conservation of natural blood products, when the need becomes overwhelming (e.g., mass casualty scenarios). The path to clinically establish such blood surrogate technologies is fraught with many challenges, including establishment of reasonably inexpensive yet efficient large-scale GMP manufacturing processes, rigorous preclinical evaluation in animal models to establish safety and efficacy profiles, obtaining FDA approval to design appropriate clinical studies, and potentially executing those studies demonstrating efficacy without any adverse effects in patients. Navigating these translational milestones requires considerable time, interdisciplinary efforts, and dedicated investment from government and private entities. Considering the fact that many hemorrhage-associated mortalities could be potentially prevented if natural blood or blood surrogate products can become readily available for in-hospital and prehospital transfusion, such investment of time, effort, and resources is of critical importance. Some researchers have also attempted to design WBC surrogates for enabling "targeted immune response," although this research has not progressed as much [240–242]. With the evolution of manufacturing processes, improved storage and distribution logistics, and refinement of DCR strategies, one can envision a future where "synthetic blood substitutes" can become complimentary to natural blood products in transfusion medicine, to allow timely treatment of hemorrhage, bleeding dysfunctions, and coagulopathy and advance toward the goal of "zero preventable death" from trauma [243], in both military and civilian settings.

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Flow-Based Coagulation and Fibrinolysis Assays

44

Matthew Sorrells and Keith B. Neeves

Introduction

The local hemodynamic environment has a profound influence on the formation and structure of a thrombus. The gross anatomy of platelet-rich "white clots" in arterial thrombosis and erythrocyte-rich "red clots" in venous thrombosis is one oft-cited example [1, 2]. At the cellular level, it is well recognized that platelet adhesion and aggregation are regulated by shear stress; different receptors play different roles in venous, arterial, and pathologic flows [3]. Perhaps not as well appreciated is the role blood flow plays in regulating the enzymatic networks of coagulation and fibrinolysis [4].

Blood flow affects coagulation and fibrinolysis in at least three ways. First, it determines the delivery rate of zymogens, anticoagulants, and other plasma proteins to a vascular injury and evolving thrombus [5–7]. Under static conditions, coagulation products must find each other or phospholipid surfaces by diffusion, which is a slow process that scales as the square root of time. Under flow conditions, the diffusion length is significantly reduced into a thin boundary layer near the vascular or other surfaces. The net effect is a continued renewal of reactants for reactions occurring on phospholipid surfaces that support coagulation like vascular cells, activated platelets, and circulating extracellular vesicles.

Second, blood flow determines the dilution rate of the products of coagulation and fibrinolysis. In this way, blood flow acts as an anticoagulant by washing away products like thrombin and fibrin monomers [8–10]. The dilution effect explains, in part, why thrombi formed at high blood flow rates are relatively fibrin-poor compared to those formed at low blood flow rates. There is an inverse relationship between platelet accumulation and fibrin formation with the former increasing, and the latter decreasing, with higher blood flow rates [11].

Third, blood flow, or more specifically the shear and normal stresses imposed by blood flow, dictates the accumulation and interaction of platelets, leukocytes, and erythrocytes with the vascular wall and each other [12–18]. This in turn determines the surfaces available for coagulation reactions and interstitial transport within thrombi. For example, highly activated platelets expose phosphatidylserine (PS), which supports several coagulation reactions and allows a thrombus to propagate beyond the vascular wall [19]. Moreover, the physical packing of blood cells determines the ability of coagulation products and reactants to transport into and out of a growing

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clot [20–22]. The dense core of a thrombus near the vascular wall is nearly impenetrable and traps products like thrombin, while the more porous shell allows for the passage of plasma proteins.

A research tool for studying coagulation under flow in vitro is a microfluidic flow assay. These assays are miniaturized versions of the parallel plate and annulus flow assays that have been widely used in cell adhesion studies since the 1970s [23]. The general procedure for these assays is to perfuse whole blood over prothrombotic substrates at a constant flow (shear) rate and record the kinetics of blood cells and fibrin accumulation [24–26]. Alternatively, these assays can be run at a constant pressure to form an occlusive thrombus [27, 28]. Coagulation can be initiated through both the intrinsic and extrinsic pathways [29–31]. Type I collagen is the most common substrate used in these assays, and it can promote the activation of factor XII (FXII), initiating the intrinsic pathway and ultimately supporting fibrin formation [32]. Tissue factor (TF) can be immobilized to the surface or added to whole blood in suspension to initiate the extrinsic pathway [33, 34]. Note in this chapter we will abbreviate coagulation proteins in their zymogen or inactive forms as FV (factor V) and their enzyme or active forms as FVa (activated factor V).

In this chapter, we review some of the recent work on flow-based coagulation assays. The first section above describes some of the mechanisms by which blood flow regulates coagulation and fibrinolysis. The second section provides an overview of the principles of flow assays. The third and fourth sections describe studies related to the influence of blood flow on coagulation and fibrinolysis, respectively. The studies highlighted in these sections are conducted in straight channels and parallel, laminar flow. The fifth section describes the design and application of devices with geometries or flow conditions that mimic various pathologies: stenotic vessels, penetrating vessel injuries, and venous valve stasis. In these examples, the hemodynamics are more complex including vorticity, shear gradients, and turbulence. In the sixth section, we review the small number of studies to date using these approaches in the context of trauma. Finally, the seventh section concludes with an outlook on the challenges for translating these assays into a clinical setting.

Overview of Microfluidic Flow Assays

Microfluidic flow assays offer a low volume and low-cost approach to modeling hemostasis and thrombosis under flow. The operating principle of these assays is to perfuse blood through a rectangular microfluidic channel over adhesive and/or procoagulant substrates [10, 35, 36]. These channels can be completely coated with these substrates, or they can be micropatterned to mimic focal injuries. For example, Fig. 44.1a–c shows a method for patterning whereby a solution is incubated on a glass slide within a small microwell leaving a circular patch of a prothrombotic substrate. Microfluidic channels are laid over the patterned substrate and blood is perfused through the channels (Fig. 44.1d, e). The microfluidic channels are typically made of transparent polymers allowing for high resolution imaging, with polydimethylsiloxane (PDMS) being the most common. Thrombus formation on the patch is observed by optical microscopy, and thrombus growth can be measured by changes in pressure drop for constant flow rate or flow rate for constant pressure drop [27, 37] (Fig. 44.1f). These devices, with dimensions that range from the arteriole (~100 μm) to the capillary (~10 μm), can accurately replicate the fluid mechanics and shear stresses present in the human microvasculature [38]. A wide variety of prothrombotic substrates [39] with tightly controlled spatial presentation [40] coupled with multichannel devices provide the ability for high content screening of thrombus formation [23].

Microfluidic devices offer several attractive features for measuring coagulation under flow but come with limitations. The main advantages of microfluidic flow assays are precise control over experimental conditions; device geometry, shear rate, flow regime, injury size, and injury composition are all user-defined inputs. Another key feature is that these devices are usually transparent, allowing higher resolution visualization and more reliable image quantification than intra-

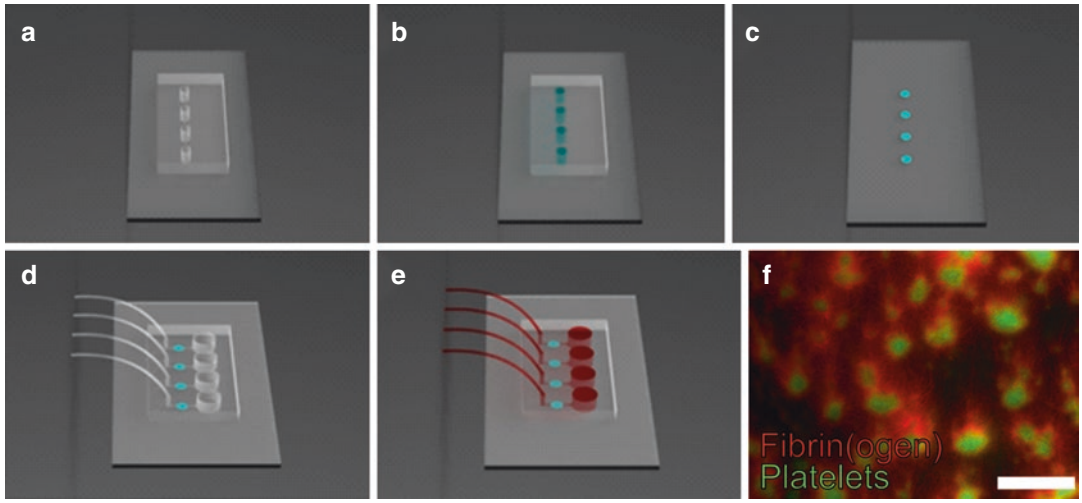


Fig. 44.1 General workflow for microfluidic flow assays. (**a**–**c**) A glass slide is patterned with prothrombotic proteins (e.g. collagen, fibrinogen, von Willebrand factor, and/or tissue factor). (**d**) A microfluidic device is placed on the glass slide such that the channels of the device run over the patterned substrate. (**e**) Blood is perfused through the microfluidic channel either using a pump or constant pressure source. (**f**) At the site of the patterned substrate, thrombus buildup is visualized using optical microscopy. Scalebar = 50 μm

vital microscopy. A limitation of microfluidic assays is that they cannot capture all of the physics of thrombus formation at the scale of large arteries and veins because one cannot match both the wall shear stress and inertial forces found these vessels in small channels [38, 41]. In addition, many studies do not incorporate endothelial cell function into the device design, removing an important player in thrombosis and hemostasis [42]. Although culturing cells inside microfluidics has become more common, and there are several examples of integrating endothelial function into flow assays [43–46]. Another difficulty that arises when incorporating coagulation is that little is known about the vascular wall concentrations of pro- and anticoagulation molecules like TF and thrombomodulin (TM) although their expression is known to qualitatively vary in different vascular beds [47, 48].

Flow-Based Models Incorporating Extrinsic and Intrinsic Coagulation Pathways

In this section we discuss studies that have used straight, rectangular channel flow assays to measure the mechanisms regulating the intrinsic and extrinsic coagulation pathways. Table 44.1 summarizes these studies on coagulation under laminar, parallel flow.

The extrinsic, or tissue factor (TF), pathway is the primary initiator of coagulation in arterial and venous thrombosis [49, 50]. When TF is exposed to blood, it binds with plasma FVII and FVIIa, to form the extrinsic tenase complex (TF:FVIIa). This complex catalyzes the zymogens FIX and FX to their activated forms, FIXa and FXa, which then bind with their cofactors FVIIIa and FVa, respectively to form the intrinsic tenase complex (FVIIIa:FIXa) and prothrombinase complex (FVa:FXa). The prothrombinase complex converts prothrombin to thrombin, a serine protease that both activates platelets through protease-activated receptors (PARs) and catalyzes fibrin polymerization. TF is found in the vessel wall and on circulating blood cells (e.g., monocytes) and extracellular vesicles [51].

Okorie et al. were the first to integrate immobilized TF and measure its surface concentration in whole blood flow assays [29]. Whole blood was perfused over an array of collagen-TF microspots while measuring platelet and fibrin deposition after 5 minutes. In agreement with predictions of Kuharsky and Fogelson's mathematical model of coagulation under flow [52], they found a thresh-

Table 44.1 Flow assay studies of the extrinsic and intrinsic pathways

Pathway	Surface	TF surface concentration (molecules/μm^2)	Shear rate (s^{-1})	References
Extrinsic	Type I collagen + TF	0–25	100–1000	[29]
Extrinsic	TF-coated silica beads	0.08–8	50–1000	[57]
Extrinsic	Type I collagen + TF	0.1–2	100	[55]
Extrinsic	Type I collagen +TF	13.9	100	[54]
Extrinsic	Type I collagen + TF	0–18	1000	[56]
Extrinsic/intrinsic	Type I collagen + kaolin or TF	–	100, 1000	[65]
Extrinsic/intrinsic	Type I collagen, type I collagen + TF	0–2	100, 1000	[31]
Extrinsic/intrinsic	Type I collagen, type I collagen + TF	0–2	100	[66]

old response to surface TF concentration that varies with shear rate; higher concentrations of TF are needed to exceed the threshold response at higher shear rates [29]. Under static conditions the response to TF is more graded [53], demonstrating a fundamental difference between flow and static coagulation assays.

Onasoga-Jarvis et al. used whole blood from individuals with hemophilia A to compare the relative roles of the intrinsic tenase (FVIIIa:FIXa) and extrinsic tenase (TF:FVIIa) on fibrin formation [54]. They compared fibrin formation from blood from healthy donors to patients with mild, moderate, and severe FVIII deficiencies on collagen-TF surfaces [54]. Significant fibrin formation was supported in blood with mild FVIII deficiencies but was not observed in moderate or severe FVIII deficiencies. However, significant differences were observed in platelet aggregate size between the clinical categories. The dynamics of fibrin generation following FVIII replacement therapy and bypass therapy through recombinant FVIIa (rFVIIa) were also compared. While FVIII replacement restored the fibrin formation to normal levels, rFVIIa bypass changed fibrin dynamics by decreasing the lag time and increasing the rate of fibrin generation. Li et al. conducted a similar study with hemophilia blood focusing solely on the coagulation activated by type I collagen. They found that rFVIIa therapy could support enough thrombin generation to enhance platelet activation, but not fibrin deposition, in severe FVIII deficiencies. This was in contrast to moderate FVIII and FIX deficiencies where fibrin deposition was also rescued [55]. This result illustrates key differences in the dynamics of thrombin and fibrin formation under flow via manipulation of the intrinsic and extrinsic tenase complexes.

Thomassen et al. used a TF-initiated flow assay to determine the effectiveness of tissue factor pathway inhibitor (TFPI) inhibition to rescue fibrin formation in hemophilia A [56]. They showed that inhibiting TFPI increased fibrin formation in normal blood only at low levels of TF concentration, but in FVIII-deficient plasma and blood from individuals with hemophilia A, TFPI inhibition caused a significant increase in fibrin formation across a wide range of TF concentrations. These results demonstrated the potential for TFPI inhibition as a means of treating hemophilia.

In an approach that does not rely on fresh whole blood, Onasoga-Jarvis et al. developed a flow assay that uses plasma and biomimetic TF surfaces [57]. Here, 800 nm silica nanoparticles coated with a TF-rich phospholipid bilayer were micropatterned into 20–100 μm islands. Plasma was perfused over these islands and the kinetics of thrombin generation and fibrin deposition were measured by fluorescence microscopy. Thrombin generation was measured indirectly through a fluorogenic substrate. Notably, lipidated TF micropatterned on a flat substrate without nanoparticles and even at very high TF concentrations did not support fibrin deposition. This observation shows the importance of the interstitial space between the silica particles in this assay, but also suggests those spaces between

blood cells in a thrombus are essential in protecting coagulation products from dilution by flow. As in whole blood, a threshold response in thrombin generation and fibrin formation to surface TF concentration was observed. Electron microscopy showed smaller diameter fibrin fibers at higher shear rates, thereby also demonstrating the dilutional effect on fibrin polymerization as well as coagulation products. FVIII- and FIX-deficient plasmas showed decreased thrombin and fibrin generation at a low TF concentration but were restored to healthy generation levels at a high TF concentration, showing that a sufficiently high TF concentration can support healthy coagulation levels in hemophilia.

The intrinsic pathway, or contact, pathway consists of the activation of coagulation factor XII (FXII) through negatively charged molecules, negative surfaces, or the kallikrein protease. FXIIa activates FXI to FXIa, which then activates of FIX to FIXa, and finally this pathway converges with the common pathway by activation of FX via the intrinsic tenase (FVIIIa:FIXa) complex [58]. Physiologic triggers of the contact pathway include polyphosphate secreted from the dense granules of platelets and neutrophil-derived extracellular DNA [59]. Individuals with FXII deficiencies do not bleed excessively, and blockage of FXII and FXI is an effective strategy for attenuating thrombosis in animal models [60, 61]. Thus, the contact pathway is an attractive target for reducing thrombosis in humans and extracorporeal devices [62], potentially without increasing bleeding risk [59, 63, 64].

Zhu et al. performed a series of studies on the contact pathway by running flow assays at 100 and 1000 s^{-1} on type I collagen with no TF or at a low TF concentration [31, 65–67]. For type I collagen alone at 100 s^{-1}, fibrin formation was observed after 5 minutes and was completely ablated through inhibition of FXI. Assays at surface TF concentrations of 0.1–2 molecules/μm^2 showed that contribution of FXIa and activation of FXII via platelet-derived polyphosphate is limited to regimes of surface TF concentration on the order of 0.1 molecules/μm^2, thereby adding to our understanding of the interplay of the extrinsic and intrinsic pathway at different extents of injury [31, 65]. Additional work showed that VWF fibers may promote contact pathway activation via FXII capture [67].

Neutrophil extracellular traps (NETs) have been linked to a variety of functions related to innate immunity and hemostasis [68, 69]. Originally, it was thought that the DNA in NETs initiates activation of the contact pathway [69, 70], though recent evidence has found that while the DNA and histone components of NETs activate the coagulation cascade, intact NETs may not activate coagulation [71]. Nevertheless, NETs are found in venous thrombi of animal models and humans [70, 72, 73]. Microfluidics have been used to study the interplay of thrombosis and NETosis. Yu et al. concluded that NETs can form in sterile thrombi at high shear rates, likely due to shear stress-induced lysis of neutrophils [74]. Furthermore, they showed that there was an inverse relationship between the degree of fibrin polymerization in flow assays and formation of NETs due to the increased shear forces experienced by neutrophils in a fibrin-poor clot versus a fibrin-rich clot [75], thus providing another piece of evidence for the potential link between NETosis and coagulation.

The discoveries reviewed in this section have been enabled by the unique features of flow assays, mainly the ability to set surface or bulk concentrations of initiators and independently manipulate wall shear rate. Defining surface TF concentration allowed for measurements of the interplay between the extrinsic pathway and shear rate [29, 57] in the context of normal and coagulopathic blood. Similarly, being able to change surface TF concentration allowed researchers to understand what ranges of surface TF concentration correspond to a significant contribution of the intrinsic pathway in coagulation [31, 65].

Flow-Based Models Incorporating Fibrinolysis

Traditional whole blood flow assays do not incorporate fibrinolysis because the primary physiologic source of plasminogen activators is endothelial cells [42]. While there has been considerable effort into building endothelial and vas-

Table 44.2 Flow assay studies incorporating fibrinolysis

Surface	Shear rate (s^{-1})	tPA (nM)	References
Type I collagen + TF	150, 300, 1000	75	[76]
Type I collagen, type I collagen + TF	200, 1222	50	[77]
Type I collagen + TF	400, 1500	140	[84]

cular wall function into microfluidic flow assays [43–46], at the time of this writing none, that we are aware of, have examined endothelial cell–mediated fibrinolysis. Rather, the flow studies performed to date incorporate fibrinolysis by adding exogenous tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA) into whole blood or buffer solutions prior to introduction into flow chambers. Table 44.2 summarizes some key studies using exogenous plasminogen activators in whole blood flow assays.

Whyte et al. added tPA or uPA at two steps during flow assays. First, they added them into whole blood before perfusing it over a type I collagen-TF surface at wall shear rates of 300 and 1000 s^{-1} [76]. Next, after forming stable thrombi, they perfused tPA or uPA in buffer and measured fibrinolysis by the loss of fluorescently labeled fibrin within and around the thrombi. They demonstrated a dose-dependent response of fibrinolysis to tPA and uPA and showed that fibrinolysis could be inhibited using tranexamic acid. Fibrinolysis was observed on a timescale of roughly 15 minutes. They reported no differences in the degree of fibrinolysis between assays at different shear rates, indicating that fibrinolysis was kinetically limited rather than transport limited from 300 to 1000 s^{-1} under these experimental conditions.

Li et al. added tPA to whole blood prior to introduction into a flow chamber [77]. These assays were performed at a constant pressure gradient rather than constant flow rate, using initial shear rates of 200 and 1222 s^{-1}, which allowed occlusive thrombi to form. Fibrinolysis was measured by comparing the degree of fibrin formation in assays with tPA to assays without tPA or assays that inhibited fibrinolysis via ε-aminocaproic acid. Using this model, they showed fibrinolysis on a time scale of 15 minutes, roughly the same as Whyte et al. [76]. Though comparable degrees of fibrinolysis were observed at both shear rates, more unstable thrombi were observed at 1222 s^{-1} as evidenced by embolization.

Loyau et al. initiated fibrinolysis in whole blood after forming a thrombus. First, a thrombus was formed with recalcified, citrated whole blood perfused over a collagen-TF surface at 400 s^{-1} or 1500 s^{-1} [78]. Platelets were labeled with one dye (3,3′-dihexyloxacarbocyanine iodide) and accumulation was monitored over 4 minutes. Next, fibrinolysis was measured by perfusing whole blood containing tPA for 10 minutes. During this step, platelets labeled with another dye (rhodamine 6G) were used to distinguish secondary platelet recruitment during fibrinolysis. Through this approach, they showed that secondary platelet recruitment during fibrinolysis is inhibited with antagonism of glycoprotein VI and P2Y12 receptors.

Flow assays allow for visualization of both fibrin deposition and degradation spatially and temporally within and around a thrombus. In each of these studies, fibrinolysis is observed on the time scale of 10–20 minutes using therapeutic concentrations of plasminogen activators. A major point of disagreement is the shear rate dependence of fibrinolysis. Li et al. observed comparable fibrinolysis at all shear rates, but thrombi formed at 1222 s^{-1} were inherently less stable than those formed at 200 s^{-1} [77]. Loyau et al. showed greater fibrinolysis at 400 s^{-1} compared to 1500 s^{-1} [78]. Whyte et al. showed no shear dependence in their assays [76]. This could be in part due to the differences in working tPA concentration used and the different methods of inducing fibrinolysis (Table 44.2).

Flow-Based Models of Pathologic Hemodynamics

Though most flow assay studies are conducted in rectangular channels, microfabrication allows for other geometries that can mimic the anatomy of vessels and fluid mechanics of various pathologies. Most studies to date and described in this section focus on platelet and von Willebrand factor (VWF) function. We have included them here

to illustrate the importance of vessel/channel geometry on thrombus formation.

Microfluidic flow assays have been integral in describing the role of shear gradients that support activation-independent platelet aggregation. This phenomenon was first reported by Nesbitt et al. in severely stenosed murine vessels and flow assays [79], and then extended by Tovar-Lopez

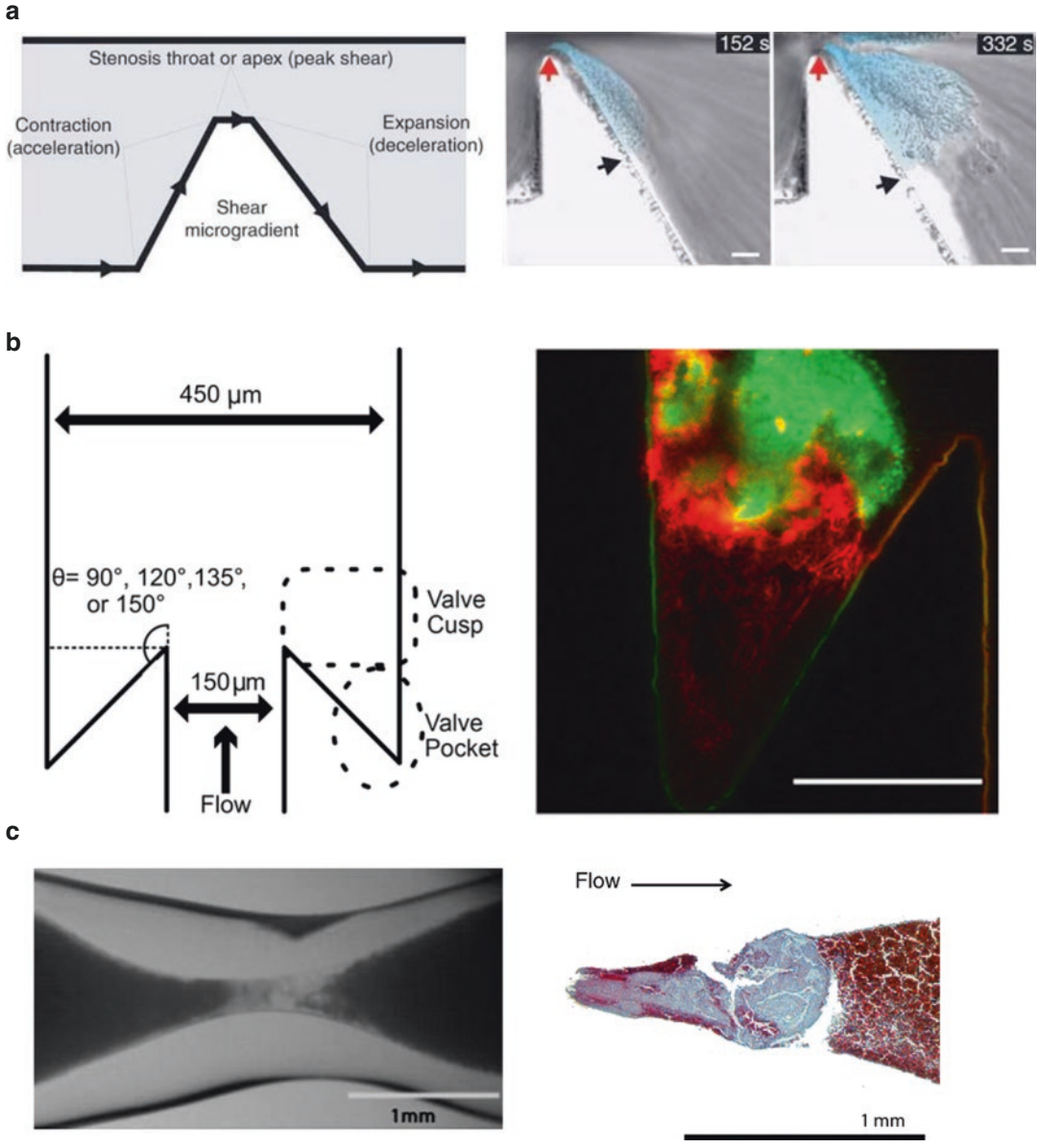


Fig. 44.2 Anatomically inspired flow assay geometries coupled to pathologic hemodynamics. (**a**) Left: Geometry of a severe stenosis in a microfluidic device for inducing shear gradients. Right: Microfluidic device of severe stenosis with platelet aggregation occurring on the downstream face of the stenosis. Scalebar = 10 µm. (Taken with permission from [79]). (**b**) Left: Geometry of venous valve for inducing secondary (vortical flows). Right: Microfluidic device of a venous valve where fibrin accumulates in the valve pocket and platelets drive thrombus propagation beyond the valve cusp. Scale bar = 150 µm. (Taken with permission from [84]). (**c**) Left: A cylindrical glass stenosis model with platelet accumulation at the throat. Right: Histology of the thrombus shows a platelet-rich "head" stained blue that formed on or just after the throat of the stenosis with a downstream fibrin and erythrocyte-rich "tail". (Taken with permission from [105])

et al. in a series of stenosis geometries (Fig. 44.2a) [80]. Westein et al. investigated the role of degree of stenosis in a model with micropatterned VWF and fibrinogen [81]. When varying extent of stenosis from 20% to 80% a sharp increase in platelet aggregation was observed. Increasing the overall length of the stenosis caused reduced platelet aggregation, demonstrating that the length along which platelets accelerate or decelerate is another important factor in this phenomenon. The platelet adhesion and aggregation in this stenosis was highly VWF-mediated as GPIb antagonism decreases and elevated plasma VWF increases platelet accumulation.

Jain et al. used a stenosis-based flow assay to create an automated point-of-care stenosis microfluidic assay [82]. The device consisted of several parallel channels that incorporated a stenosis with type I collagen or no protein coating. Thrombi formed predominantly in the poststenosis region of the device where flow decelerates. Thrombus formation was observed both on bare surfaces and with type I collagen and was sensitive to integrin $\alpha_{IIb}\beta_{3,}$ thromboxane A2, and FX inhibition.

Colace et al. used a microfluidic device with severe contraction and expansion to examine the unfolding, elongation, and deposition of VWF under pathological shear rates [83]. Whole blood or platelet-free plasma (PFP) was perfused through the collagen-coated stenosis at shear rates varying from 3,000 to 125,000 s^{-1}. They showed that the unfolding and elongation of VWF was not dependent on elongational flows but rather a sufficiently high shear rate (>30,000 s^{-1} in PFP). Additionally, no difference was observed in VWF deposition between the inlet of the stenosis, where the flow is accelerating, and the outlet of the stenosis, where flow decelerates. The process of VWF aggregation was directly influenced by calcium ion concentration, in which the absence of calcium ions resulted in the formation of longer VWF fibers. Experiments where whole blood was first perfused at 200 s^{-1} to promote platelet accumulation to collagen and then raised to 7800 s^{-1} showed that previously bound platelets enhance VWF deposition at high shear rates.

Lehmann et al. simulated some of the salient hemodynamics of deep vein thrombosis in a device consisting of two symmetrical model venous valves and TF-driven thrombus formation (Fig. 44.2b) [84]. Two countercurrent vortices were observed within each valve sinus at sufficiently high flow rates, matching observations in the venous valves of dogs and humans [85]. Thrombus formation followed a two-stage process: First, fibrin and red blood cells (RBCs) accumulate in the deepest recesses of the valve sinus where a low flow secondary vortex exists. Second, platelets adhere to the fibrin fibers at the interface of the primary and secondary vortices and propagate the thrombus out of the sinus and into the bulk flow. RBCs were necessary to drive platelets to the fibrin interface in the valve pocket. Glycoprotein VI (GPVI) signaling was necessary for platelets to become procoagulant and support thrombus growth beyond the valve sinus.

In addition to microfluidic assays, important work has been done in to-scale flow models to characterize thrombosis in large vessels [58]. Ku and Flannery used a cylindrical 1.5 mm diameter glass stenosis model to study the dynamics of thrombus formation of aortic stenosis (Fig. 44.2c) [86]. The model was coated with type I collagen, and heparinized porcine blood was perfused through the stenosis at initial shear rates from 10,000 to 40,900 s^{-1}. The histology of thrombi formed in this device showed a platelet-rich head of the thrombus near the throat of the stenosis and fibrin and a VWF-rich tail of the thrombus downstream from the stenosis. Bark et al. performed studies in a similar device in which image analysis was done to calculate the precise thrombus growth rate and shear rate throughout the stenosis [87]. They observed a maximum thrombus growth rate at approximately 6000 s^{-1}. Though the blood volume required for this experimental setup was quite large (100–250 mL), it likely better represents large artery thrombosis due to the cylindrical geometry and significant inertial forces present at this length scale and flow rate.

In these studies, the control over geometry allows for the recreation of hemodynamic features of arterial and venous thrombosis. Expansions and contractions led to gradients in

the velocity field that result in important biophysical phenomena including activation-independent platelet aggregation, vortical flows that support platelet adhesion and coagulation, and shear and elongational stresses that induce conformational changes in VWF.

Flow-Based Models of Bleeding

The devices described in the previous sections focus on intravascular thrombus formation that is relevant to superficial injuries of the vessel wall or arterial and venous thrombosis, that is, thrombi form within the vessel. Bleeding is different in that a penetrating injury causes blood to be in contact with the extravascular environment, which is biochemically and biophysically distinct from the intravascular environment. Devices designed to model bleeding are typically performed at constant pressure drop, rather than constant flow (shear) rate, across a model injury to allow for the formation of a platelet plug to stem blood loss. Table 44.3 summarizes these studies.

Muthard et al. developed a microfluidic model that incorporated a "T" geometry where a side channel perpendicular to a main flow channel contains type I collagen and TF [88] (Fig. 44.3a). A constant pressure gradient was set between the inlet and outlet of the side channel. Increasing the pressure gradient resulted in different clot morphologies. Higher pressure gradients gave smaller clots with lower permeabilities than those formed at lower pressure gradients. A tightly packed core of highly activated platelets surrounded by a more porous, less activated shell of platelets was observed, similar to the thrombus morphologies described in a laser injury model in murine cremaster vessels [22]. This device was used to investigate the role of reduced blood flow on clot retraction [89]. In these experiments, a thrombus formed in whole blood was rinsed with a calcium ion–containing buffer at the same fluidic conditions, and flow was either reduced or stopped in the device. Clot retraction was then measured following the cessation of flow. The rate of clot retraction rapidly increased following the stoppage of flow, peaking at 1–2 minutes and then nearly halting at 6–7 minutes. The degree of clot retraction was dependent on adenosine diphosphate (ADP) and thromboxane A2 (TXA2)-driven platelet activation and myosin function in platelets.

Schoeman et al. developed a microfluidic model that incorporated a "H" geometry (Fig. 44.3b) [90]. Two vertical channels representing blood and extravascular compartments are connected by an injury channel coated with type I collagen and TF. By setting appropriate flowrates in the vertical channels, a constant pressure drop is achieved across the horizontal injury channel, resulting in blood flow from the blood channel, through the injury channel, and out into the extravascular channel that contains buffer. The device yields a pattern indicative of the traditional view of hemostasis; first a platelet plug forms to stanch bleeding, and then fibrin polymerizes to mechanically reinforce the clot. By tracking the time to occlusion of the injury, a clot time of 7.5 ± 1.6 minutes was reported which is on the same order of bleeding times in murine tail

Table 44.3 Flow assay models of bleeding or hemostasis

Surface	Injury dimensions (μm)	Shear rate (s^{-1})	Pressure drop across injury (kPa)	References
Type I collagen + TF	*L*: 250, *W*: ~240 (entrance) & 50 (exit), *H*: 60	174, 1130	1.2; 3.92	[88]
Type I collagen + TF	*L*: 150, *W*: 50, *H*: 20	10,900	0.68	[90]
Type I collagen + TF	*L*: 200, *W*: 50, *H*: 100	~150	–	[93]
Endothelial cell injury on type I collagen + fibronectin	*W*: 132.49 ± 40.19, *H*: 6	~300, 1000	~ 0.1	[94]

L = length, *W* = width, *H* = height

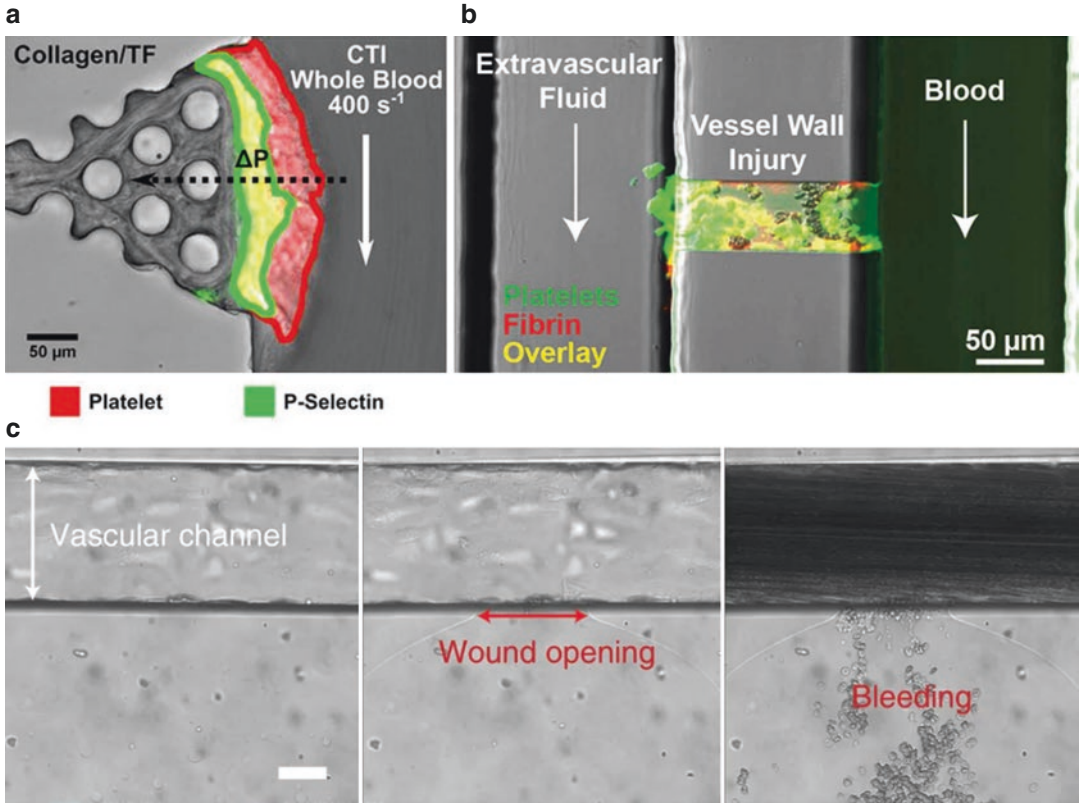


Fig. 44.3 Microfluidic models of bleeding. (**a**) A "T" geometry with a triangular side channel inlet coated with type I collagen and TF. A dense P-selectin core of platelets is surrounded by a porous platelet shell. (Taken with permission from [106]). (**b**) A "H" geometry with the horizontal channel coated with type I collagen and TF. A platelet plug, stabilized by fibrin, stops blood loss from the "blood" channel to the "extravascular" channel. (Taken with permission from [90]). (**c**) An endothelialized microfluidic channel with a trap-door mechanism that creates an injury to the channel through which blood escapes and then forms a hemostatic plug. Scale bar = 50 μm. (Taken with permission from [94])

bleeding models (1.5–8 minutes, depending on the technique) [91, 92]. The model is sensitive to both platelet function and coagulation based on studies using a P2Y12 antagonist and FVIII inhibition.

Though done on a constant flow rather than constant pressure setup, Zilberman-Rudenko et al. performed experiments and simulations on thrombus formation in a complex bypass network [93]. This type of network is comparable to the vessel topology in the microvasculature. The device consisted of a ladder-type network with a vertical inlet channel connected to four horizontal, parallel channels arranged as "rungs" on the ladder that all converge at a common vertical outlet channel. The whole device was patterned with type I collagen and TF, thereby supporting both platelet adhesion and aggregation and coagulation. Experiments run in the device showed an increasing degree of thrombus formation in each subsequent "rung" of the ladder network. Simulations that analyzed changes in flow and advection of agonists in response to obstructions in the ladder network explain this finding. Simulations showed that at the beginning of the assay, flow was primarily through the first rung of the ladder, causing occlusion to happen there first. However, when the first rung of the ladder occluded, the flow rerouted primarily to the second rung and contained thrombin advected downstream from the first rung, causing increased thrombus formation. This process repeated to

each subsequent rung until each occluded, the last of which contained the most thrombin due to the increased advection of thrombin from the upstream thrombi, causing increased thrombus formation.

Sakurai et al. incorporated endothelial cells into a microfluidic model of bleeding (Fig. 44.3c) [94]. Here, a valve-actuated trap door opens, disrupting the endothelial layer in one channel and diverting blood through a second outlet channel where it is exposed to type I collagen and laminin. Bleeding time was used as a benchmark for the hemostatic response of the system. Prolonged bleeding time was observed upon inhibiting von Willebrand Factor (VWF)-platelet binding, and prolonged bleeding time and reduced fibrin were found when using FVIII-deficient or -inhibited blood.

The collection of microfluidic models of bleeding is in early development. Both Schoeman et al. and Sakurai et al. show that their models are sensitive to platelet function and coagulation. Zilberman-Rudenko et al. demonstrated the complex dynamics of thrombus formation in vessel networks. Muthard et al. validated the physiological relevance of their model by comparing platelet buildup and P-selectin distribution of their model to that of a mouse cremaster injury model [22]. More work is needed to show whether these models provide distinct information about bleeding diatheses that is not available with conventional flow assays or existing clinical measures of bleeding risk.

Flow-Based Models of Trauma

Only a small number of studies have used flow assays in the context of trauma; they are briefly described here and summarized in Table 44.4.

In conjunction with thromboelastometry (TEG) and in vivo experiments, Wiener et al. ran whole blood flow assays on type I collagen at 100 s^{-1} with 100 μM taurocholic acid, a substance released during shock in animal models of trauma [95]. Blood incubated with taurocholic significantly inhibited platelet function in these assays, introducing a potential pathway of trauma-induced coagulopathy.

Table 44.4 Flow assay studies of simulated trauma or trauma patients

Surface	Shear rate (s^{-1})	Treatment or patient group	References
Type I collagen	100	100 μM taurocholic acid	[95]
Type I collagen, type I collagen + TF	100, 200, 1222	Hemodilution or trauma patient samples	[77]
None (platelet aggregation mediated through high shear gradients)	~10,000	Trauma patients with and without transfusion	[97]

Lawson et al. compared TEG measurements with the results of the commercial T-TAS flow assay [96]. A cohort of healthy controls and patients with pathologies not associated with coagulopathy was used in TEG and T-TAS assays. T-TAS assays were both performed with type I collagen to measure platelet function, and type I collagen and TF to measure platelet function and coagulation. Only a few weak correlations were found between TEG and T-TAS parameters.

Li et al. used blood samples from trauma patients in flow assays on type I collagen to measure changes in platelet function. Most trauma patients showed a loss of platelet function relative to a healthy control, and of the seven patients requiring transfusion, four showed decreased platelet buildup.

Ting et al. developed a device that initiates platelet aggregation by shear gradients and measures contractile forces of thrombi [97]. This device was used to measure the contractile forces of aggregates formed from blood samples of trauma patients. Significantly higher contractile forces were observed in healthy donors and trauma patients not requiring transfusion than those that did require transfusion. When comparing these results with clinical assays they found the contractile forces measured in their devices correlated with maximum clot firmness in TEG and ADP- and arachidonic acid–induced platelet aggregation.

There are other studies done with flow assays that, while not directly done in the context of trauma, may be relevant to trauma. A major source of coagulopathy during trauma is hemodilution. The margination of platelets by erythrocytes to the near wall region is a well-documented biophysical phenomenon; they push platelets toward the outer sections of the blood vessel, thereby concentrating platelets at the near wall region of the blood vessel. Tangelder et al. described this phenomenon by quantifying the distribution of fluorescently labeled platelets or platelet-sized particles in rabbit arterioles [98, 99]. Aarts et al. later used a high resolution laser-Doppler velocimetry to quantify the distribution of platelets in a 3 mm diameter glass tube [100]. Together, these studies showed that erythrocytes marginate platelets to increase the concentration of platelets in the near wall region of vessels, and this phenomenon is more pronounced at higher shear rates. Studies that have looked at platelet adhesion to collagen in the presence of erythrocytes show increased platelet adhesion nearly 60-fold on the subendothelium compared to platelets alone [17, 101].

The effect of platelet count on thrombus formation has also been measured in flow assays. Brazilek et al. examined the effect of platelet count on thrombus formation in vitro by varying the concentration of platelets from 25 to 200×10^9 L^{-1} at a fixed hematocrit by combining citrated suspensions of washed erythrocytes, platelet-rich plasma (PRP), and platelet-poor plasma (PPP) in varying combinations [102]. Assays were run over a stenosis coated with VWF, with shear rates on the order of 20,000 s^{-1}. At platelet counts at or below 50×10^9 L^{-1}, little to no thrombus formation was observed. From concentrations from 50 to 200×10^9 L^{-1} a linear increase in thrombus formation was observed. Lisman et al. examined the effect of platelet count on thrombus formation by reconstituted platelet counts in a straight channel, laminar flow assay [103]. Assays were run at 1600 s^{-1} over a type III collagen and fibrinogen surface. At platelet counts greater than 100×10^9 L^{-1}, little to no increase in thrombus formation was observed, showing that at sufficiently high platelet counts the dynamics of platelet buildup are limited by the kinetics of platelet binding rather than the concentration of platelets. The discrepancy of these two studies, where the former shows a linear effect of platelet count and the latter shows no dependence at normal platelet counts, may be explained through the differences in geometry and adhesive substrate.

The number of flow assay studies in trauma to date is relatively small. Some work has been done to demonstrate the effectiveness in using flow assays for potentially screening for the need for transfusion and comparing it to other clinical assays. The effects of hemodilution on thrombus formation have been studied, but not in combination with other complications associated with trauma like hyper- and hypofibrinolysis.

Translating Flow Assays to the Clinic

Flow-based coagulation and fibrinolysis assays have yet to be adopted for clinical use. A number of custom and commercial systems are available; however, there are no large clinical studies that show an advantage of flow assays over existing clinical assays. There are features of microfluidic systems that could make them attractive as either point-of-care or laboratory assays. Low blood volume requirements, as little as 50 μL, is a major advantage of microfluidic assays for work with neonates or other clinical situations where large blood volumes are prohibitive. Additionally, as we have reviewed in this chapter, unique geometries, fluid dynamics, and surface conditions of microfluidic devices have the potential to screen for specific mechanisms of flow-based regulation of coagulation and platelet function. Most flow assays measure global platelet function and coagulation. As such, they are most likely to find application as screening, rather than diagnostic, tools to assess bleeding or thrombotic risk.

The potential for flow assays as a clinical assay has been best shown in the context of VWF deficiencies. Lehmann et al. used a microfluidic flow assay patterned with type I collagen to distinguish type 1 von Willebrand disease (VWD), low VWF, mucocutaneous bleeding of unknown origins, and healthy controls. They report a positive correla-

tion between patient VWF levels and platelet aggregation [104]. The PFA-100, a simple commercial flow assay, showed similar trends but with less variance between the different clinical categories. Brazilek et al. performed a similar study using a high-shear stenosis device [102]. The device was capable of differentiating aggregation levels of healthy controls from these patients with different subtypes of VWD as well as patients with low levels of VWF that were on the threshold of being classified as type 1 VWD. The assay output showed a direct correlation with measured concentrations and activity levels of VWF in patients and showed an inverse correlation with clinical bleeding scores. The device showed a similar ability to differentiate VWD from healthy controls as the PFA-100. Nogami et al. performed a study comparing the flow assay outputs to VWF activity measurements in their ability to predict bleeding scores [37]. The assay showed a better correlation with VWF level activity and bleeding score measurements when ran in the absence of coagulation. Combining the outputs of the assay with the VWF activity assay increased the overall predictive ability for bleeding scores.

Conclusion

In this chapter, we discussed how blood flow regulates coagulation and how microfluidic flow assays have been used in preclinical and mechanistic studies. The examples presented demonstrate the ability to tune the biochemical and biophysical environments to understand interactions at play between hemodynamics, platelet function, coagulation, and fibrinolysis. The relative roles of the extrinsic and intrinsic coagulation pathways on thrombus growth and stability have been determined by varying the shear rate and composition of prothrombotic substrates. The control of device geometry has allowed for the creation of devices that are designed to mimic the forces and flows in anatomically specific thrombotic or hemostatic events. As these flow assays develop, we are likely to see a combination of some of the approaches we discussed to be used to further map the interplay of biophysics and biochemistry that occurs during thrombus formation under flow. In the context of trauma, these assays are sensitive to blood cell counts, platelet dysfunction, and coagulopathies, suggesting they could be useful for quantifying subsets of trauma-induced coagulopathies or identifying individuals in need of transfusion. Whether flow assays provide additional or complementary information to existing assays used in trauma requires further study.

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Animal Models of Coagulopathy

45

Daniel Lammers and Matthew J. Martin

Introduction

Trauma remains a leading cause of death worldwide and can result in a multitude of complex metabolic and physiologic changes within the body that can range from adaptive to severely maladaptive or even fatal. These changes often lead to sepsis, coagulopathy, and multiorgan failure and have been shown to directly correlate with the degree of the initial hemorrhagic shock and injury [1–4]. Trauma-induced coagulopathy (also called the acute coagulopathy of traumatic shock) is believed to be the consequence of a complex pathophysiologic interplay between derangements encountered within the intrinsic and extrinsic coagulation pathways, platelet dysfunction, endothelial dysregulation, and hyperfibrinolysis [5]. While these factors all represent the physiologic response to traumatic insult, acute blood loss, sympathetic over-activation, and other extrinsic factors such as hypothermia, acidosis, and hemodilution have been associated with worsening of the ongoing coagulopathy and resistance to attempts at correction [1, 6, 7]. Techniques such as warmed fluids, damage control surgical and resuscitation practices, administration of coagulation factor-rich substances, and prevention of hyperfibrinolysis through the administration of tranexamic acid are all currently utilized to prevent any worsening of the coagulopathy and minimize any iatrogenic injury following trauma [2, 8–10]. Despite these measures, severe trauma and shock can result in a refractory coagulopathy causing a high expenditure of blood and blood products to maintain hemostasis and adequate perfusion. The vast amounts of blood products utilized in these scenarios not only bring forth the increased morbidity associated with multiple transfusions but also a heavy logistical and financial burden on the healthcare system [11, 12]. As such, there is an urgent need to develop novel ways to diagnose, differentiate, and effectively treat trauma-induced coagulopathy.

Trauma-induced coagulopathy remains difficult to study in the clinical setting as trauma patients are often inflicted with a variety of injury patterns resulting in difficulty finding a truly homogenous subset of patients. In addition, the emergent and often chaotic nature of the initial evaluation and treatment of severely injured and bleeding patients makes performing methodical and high-quality prospective human research difficult. The multiple confounding factors and biases from the multitude of retrospective and observational prospective human studies often result in data that is difficult to extrapolate causality among broad patient populations.

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Moreover, while large database reviews may be ideal to determine associations, these studies often lack the granular detail accounted for among large, prospective, randomized controlled trials. As the scientific community continues to elucidate the pathways and mechanisms responsible for the acute coagulopathy of trauma through basic science studies, many of the novel details and lab values found to be connected to trauma-induced coagulopathy have not been previously investigated thus highlighting the need for well-planned, prospective study. Furthermore, individual physiologic response differs to varying degrees among the population. Currently, there is no predictable pattern or test to forecast a patient's physiologic response to their specific injury. For instance, data supports that roughly 18% of patients requiring a massive transfusion result in a hyperfibrinolytic phenotype, while fibrinolytic shutdown occurs in 64%, and a normal physiologic fibrinolysis is displayed in the remainder of patients [13, 14]. These varying phenotypes may significantly impact the efficacy and response to a given treatment therefore a keen understanding of the underlying physiologic processes is needed. As much of the underlying mechanisms causing the varying physiologic responses remain yet to be elucidated, it remains clear that further basic science research on the pathways and mechanisms involved with trauma-induced coagulopathy remains a crucial component toward improving the morbidity and mortality among these critically ill trauma patients.

Although human hemorrhagic shock would be the ideal model to study traumatic coagulopathy and its reversal, animal models represent a well-validated approach that allows for control over the multiple confounders seen in clinical studies. A multitude of animal models have been utilized in the past focusing on different species and model development strategies. As such, a deep knowledge base of the previous preclinical work performed remains essential to assure that the optimal model based on prior work is being utilized and that unnecessary duplication of research is minimized. Furthermore, the study intent should remain a priority in choosing a model development strategy. This will help assure the desired results are not only feasible but also clinically translatable. In general, small animal models may be ideal for studying specific pathways and mechanisms associated with a focused research question, while larger, more robust animals may prove superior for survival models and assessments of complex pathophysiologic interactions. Performing a complex experiment on a suboptimal model will result in nonideal, possible erroneous results, and therefore the importance of taking all previous data and models into account cannot be overstated. Current experimental practices utilize a multistep approach often taking many years in order to translate basic science data into clinically meaningful human results. As such, this multiyear process often begins by utilizing in vitro studies which then progress to both small and large animal studies prior to performing any human clinical trials. This chapter seeks to explore the various animal models and variables that must be considered prior to embarking in preclinical research assessing trauma-induced coagulopathy.

Model Development

Choice of Species

During the background research and protocol development periods, one should carefully assess which species will provide the optimal model. Preclinical and translational research revolving around trauma-induced coagulopathy has largely focused on large animal models due to the complex physiologic interplay associated with the acute coagulopathy of trauma. Previous animal coagulopathy models have assessed macaque, sheep, dog, swine, rabbit, rat, and mice models [15–18]. Porcine models have governed much of the trauma-specific research largely due to their relative similarities to human anatomy and physiology. Furthermore, swine models are easily obtained and can provide a

robust platform for both short-term non-survival and prolonged survival studies. However, swine remain relatively expensive and logistically difficult due to special housing and veterinary support requirements, particularly for any model that involves post-intervention survival and continued study of the animal. Small animal models such as mice and rats, on the other hand, provide a significantly cheaper and less logistically burdensome model. This results in the ability to rapidly perform the desired experiments on a large number of animals and to perform prolonged survival study with significantly less logistical and personnel requirements compared to porcine or other large animal models. These models are more suited for specific and targeted basic science research assessing unique pathways and mechanisms involving coagulopathy compared to their large animal counterparts. Despite being ideal for studying very specific pathways and mechanisms, their substantial difference in blood volume creates a difficult limitation in studying a multitude of biomarkers and proteins associated with coagulopathy over periods of time. While repeated measures of serum markers may significantly alter the animal's blood volume and affect the model, it is important to keep in mind that the catalog of validated antibodies available is vastly more robust within rodent species with only limited availability specifically designed for large animal models. Although there is a focus on the validation of newer laboratory tests for large animal models, historically the desired tests have been performed on various laboratory kits not designed for the model in question due to the scarcity of validated approaches for many particular species (i.e., various ELISA cytokine testing on porcine models have been performed using human cytokine kits). While this is not the ideal scenario, the clinical and genetic similarities among the species often impose a minimal effect and are not felt to significantly alter the results. If this testing approach is required, astute diligence is warranted to assure that interspecies differences have not previously demonstrated incompatibility or spur erroneous results.

Differences in Species

Baseline coagulation characteristics between species need to be carefully assessed prior to starting any experiment. Careful assessment of the relationship between the chosen species and human coagulation function needs to be scrutinized when attempting to optimize the experimental model (see Table 45.1). While much of the coagulation system has been relatively conserved among Mammalia, differences among various species can been seen and have been reported within the literature. As such, the unique differences among the various models may result in serious limitations and invalidity of the resultant data (in terms of translation to humans) when improper models are utilized to assess the proposed research question.

Differences in Gender

Animal studies historically have utilized male animals and cell lines to minimize variables between the sexes. Some argue that previously collected data from studies using only a single sex may not be widely applicable and does not appropriately consider the key hormonal influences between genders. The National Institutes of Health now supports the notion of including both sexes in the experimental design (or justification for the performance of single-gender studies) as this may improve the generalizability to the overall population [40]. Albeit, there remains a multitude of human data describing key sexual differences in the coagulation systems [41]. This is mainly theorized to be secondary to the differences in circulating hormone levels and suggests women may be more prone to post-traumatic hypercoagulability compared to their male cohorts [42, 43]. These concepts highlight that researchers should carefully inspect their targeted population (i.e., general population versus military population) as incorporating a single-gender cohort, despite guidance to include both sexes, may be more applicable to certain research questions proposed. Finally, if a study is using

Table 45.1 Key hematologic and hemostatic values and related assays in common laboratory animal species and humans

Parameter	Human (adult)	Swine	Sheep	Rat	Mouse
Blood volume (ml/kg)	68–88 (plasma vol) [19] 55–75 (RBC mass) [19]	56–69 [20]	58–64 [20]	54–70 [20]	60–75 [20]
Platelet count (10 [9]/L)	159–376 [19]	350–700 [20]	401 (132–650) [16]	500–1300 [20]	1000–1269 [21, 22]
Coagulation factors					
vWF (%*n*)	92 (50–158) [23]	140 [24]	117 (30–186) [16]	49 [25]	15 [21]
II (%*n*)	110 (78–138) [26]	65 (58–74) [27]	40 (36–43) [27]	110 (92–122) [27]	–
V (%*n*)	118 (78–152) [26]	790 (555–1020) [27]	62 (48–75) [27]	749 (512–781) [27]	292 [28]
VII (%*n*)	129 (61–199) [26]	58 (42–77) [27]	36 (24–50) [27]	471 (403–586) [27]	–
VIII (%*n*)	160 (52–290) [26]	555 (380–745) [27]	817 (427–1327) [16]	116 [25]	224 [28]
IX (%*n*)	130 (59–254) [26]	386 (300–590) [27]	208 (184–224) [27]	28 (24–37) [27]	75 [28]
X (%*n*)	124 (96–171) [26]	92 (59–120) [27]	15 (13–21) [27]	48 (33–59) [27]	109 [28]
XI (%*n*)	112 (67–196) [26]	200 (132–270) [27]	15 (12–18) [27]	35 (18–49) [27]	–
XII (%*n*)	115 (35–207) [26]	747 (670–840) [27]	140 (85–243) [16]	447 (312–512) [27]	–
Anticoagulation factors					
AT (%*n*)	96 (66–124) [26]	101 (89–111) [27] 101 (98–103) [29]	93 (74–119) [21]	123 [25]	–
aPC (%*n*)	103 (54–166) [26]	36 (33–39) [29]	49 (30–76) [16]	–	–
Assays					
PT (s)	13.0 (11.5–14.5) [26]	11.4 (9.4–13.1) [27]	13.1 (11.4–15.5) [16]	12.1 (8.9–13.9) [27]	7.3 [30]
aPTT (s)	33.2 (28.6–38.2) [26]	16.6 (13.4–18.1) [27]	29 (19.6–40.8) [16]	64.9 (46.2–76.1) [27]	22 [30]
Fibrinogen (g/L)	3.1 (1.9–4.3) [26]	3.57 (3.38–3.77) [29]	2.61 (1.4–4.3) [16]	1.34 [25]	–
D-dimers (μg/mL)	<0.50 [31]	<0.01 [32]	<0.02 [33]	0.18 [34]	<0.02 [34]
TEG (kaolin)					
R (min)	3.8–9.8 [35]	2.2–3.0 [36] 5.9 (5.4–6.7) [37]	–	1.0–3.4 [36]	0.9–2.1 [36]
K (min)	0.7–3.4 [35]	0.8–0.8 [36]	–	0.3–1.1 [36]	0.6–1.0 [36]
Angle (degrees)	47.8–77.7 [35]	64.8–81.6 [36] 72.4 (71.5–77.9) [37]	–	77.8–86.2 [36]	80.2–84.2 [36]
MA (mm)	49.7–72.7 [35]	80.3 (75.6–82.1) [37]	–	–	59.3 [38]
ROTEM (native)					
CT (s)	595 (476–901) [39]	244 (146–296) [39]	494 (344–1431) [39]	207 (63–352) [39]	–
CFT (s)	200 (104–436) [39]	52 (30–84) [39]	182 (143–532) [39]	55 (35–97) [39]	–
MCF (mm)	58 (49–65) [39]	74 (68–79) [39]	72 (61–77) [39]	75 (70–81) [39]	–
ML (%)	21 (2–24) [39]	17 (12–31) [39]	2 (0–26) [39]	8 (3–13) [39]	–
Cost (USD)	–	200	220	55	45

Table adopted from the 1st edition of Trauma Induced Coagulopathy (Chapter 34, Animal Models of Coagulopathy, Table 34.1; Bambakidis T, Sillesen M, Alam HB)

Mean with range is provided where available. Cost is based on approximate purchase and shipping cost available at the University of Michigan

vWF von Willebrand factor, *AT* antithrombin, *aPC* activated protein C, *PT* prothrombin time, *aPTT* activated partial thromboplastin time

sex as a key biologic variable to assess for differences in gender-based outcomes, it is important to note that the experimental arms may require an increased number of subjects per arm to be adequately powered.

Creation of Coagulopathy

The multitude of animal coagulopathy models described within the literature suggest that there are no perfect models. Table 45.2 lists a sampling of multiple studies that attempted to create a relevant coagulopathy model using a variety of animal species, degrees of hemorrhage, associated injuries, and other interventions. Pre-intervention variability among researchers and institutions further complicate the process and cloud the results by creating challenges in direct comparisons between studies due to model variations. Validated coagulopathic animal models can range from those with isolated injuries to complex and multisystem trauma. While the polytrauma models mimic a more clinically translatable picture reliant on the sophisticated interplay between an induced hemorrhagic shock, soft tissue or organ damage, and/or traumatic brain injury, variability in animal response tends to increase with multifaceted injury patterns. Furthermore, these multi-injury models supply a multitude of variables that need to be accounted for and also raise questions about the appropriate sequencing of events or interventions that contribute to the coagulopathy. Whether targeting an isolated injury pattern or performing a complex polytrauma model, the goal of obtaining a robust inflammatory response resulting in a subsequent consumption and deactivation of coagulation factors and platelets remains the same. The end coagulopathic response is often further exaggerated through a hemodilution of coagulation factors and platelets via large volumes of crystalloid administration by the research team during the procedures. As such, models will often undergo a series of interventions and stressors that ultimately lead to a common coagulopathy prior to the initiation of the study, and therefore it remains rare to associate the coagulopathic response to an isolated event within the model.

Although the goal is to create a clinically translatable model to mimic human physiology, the unpredictable nature of the disease and varying injury patterns associated within human trauma provide a daunting task. As such the spectrum of model complexity ranges widely and will be discussed in future sections.

Determination of Coagulopathy

Assessing the degree of induced coagulopathy remains a crucial data point that should not be overlooked. In human medicine and animal models, two major testing strategies are utilized in determining a coagulopathic state: conventional coagulation tests (prothrombin time in seconds (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT)) and viscoelastic testing (thromboelastography (TEG) and rotational thromboelastometry (ROTEM)). While conventional coagulation tests have remained the mainstay determinate of coagulopathy status throughout the years, recent data in support of TEG and ROTEM argue for improvements in coagulation assessment due to a more rapid precise visualization of the individual's coagulation phenotype. Both rodent and swine models have been approved for TEG and ROTEM and have commercially available reagents [29, 39, 53]. Moreover, TEG and ROTEM offer the advantage of providing a more rapid, visual representation of an individual's coagulation status compared to PT, aPTT, and INR. Furthermore, ROTEM and TEG offer the theoretical clinical advantage toward providing a product-specific resuscitation during ongoing blood loss and shock. Despite this, the current clinical standard of care still focuses on a resuscitation strategy utilizing a balanced amount of packed red blood cells, fresh frozen plasma, and platelets [10]. As more studies are performed to assess resuscitation practices favoring blood

Table 45.2 Summary of multiple published animal models of traumatic coagulopathy

Authors	Species	Hemorrhage (type and amount)	Additional injuries	Pharmacologic agents	Hemodilution	Coagulopathy model	Coagulation parameter results
Letson et al. [44]	Rodent	Controlled, fixed-pressure hemorrhage to MAP 35–40 mm Hg for 60 minutes	None	None	None	Hemorrhage	Coagulation values noted by aPTT and PT were significantly elevated 3.8-fold from baseline as early as 20 minutes post-hemorrhage
Hayakawa et al. [45]	Rodent	None	None	Tissue factor (TF) infusion over 4 hours	None	Injury-mimetic	Fibrinogen degradation products, platelet consumption, and increasing PTT were demonstrated to correspond to amount of TF infusion
Zhao et al. [38]	Rodent	None	Cecal ligation and puncture (CLP)	None	None	Intraperitoneal sepsis	Significantly increased K, decreased alpha angle, decreased MA in CLP versus sham cohorts
Donahue et al. [46]	Rodent	None	Blunt head injury	None	None	Isolated TBI	Platelet studies demonstrated decreases in platelet stimulation toward ADP, arachidonic acid, and collagen following blunt TBI compared to uninjured animals
Giannoudis et al. [47]	Sheep	Controlled fixed-pressure hemorrhage to MAP 50 mm Hg for 120 minutes	Thoracotomy ± lung contusion	None	Lactated Ringer's infusion at adequate volumes to restore baseline blood pressure	Isolated injury	Antithrombin III and factor V significantly decreased in sham animals following femoral fracture fixation compared to their baseline. This response was amplified when lung contusion and hemorrhagic shock were added
Kheirabadi et al. [48]	Rabbit	Controlled 50% blood volume during hemodilution blood exchange	Splenic laceration with 10-minute bleed time	None	Hextend versus dextran 70 versus 5% albumin	Hemodilution + intra-abdominal hemorrhage	Coagulopathy was significantly induced in all cohorts as evidenced by both conventional coagulation assays and thromboelastography values

Alam et al. [49]	Swine	Controlled fixed-pressure hemorrhage to MAP of 25–30 mm Hg for a total 60% blood volume and subsequent 30-minute shock period	Femur fracture and associated soft tissue injury. Induced hypothermia. Grade V liver injury	None	Normal saline at three times the volume of hemorrhage	Hemodilution + polytrauma	Coagulopathy noted in animals following the administration of crystalloids as evidenced by increased PT, PTT, INR, and TEG analysis
Sillesen et al. [50]	Swine	Controlled 40% blood volume with 120-minute shock period	TBI via computer-controlled cortical impact	None	None	Hemorrhage + TBI	Immediate and sustained activation of the coagulation cascade with significant increase in ELISA-based markers of inflammation and cell death
Kiraly et al. [51]	Swine	Uncontrolled intra-abdominal hemorrhage for 30 minutes	Standardized grade V liver injury	None	Lactated Ringer's or normal saline at three times the weight of the removed spleen	Hemorrhage + hemodilution	The normal saline resuscitation group was significantly more acidotic, had greater blood loss, longer PTT and PT times, and a decreased hypercoagulability during early trauma compared to the lactated Ringer's group.
Moe et al. [52]	Swine	Controlled 35% blood volume	Supraceliac aortic occlusion for 50 minutes	Epinephrine infusion upon reperfusion	None	Hemorrhage + ischemia-reperfusion model	Significantly worse acidosis, elevated base deficit, prolonged PT, decreased EXTEM MCF, and decreased fibrinogen levels

product-specific replacement through TEG and ROTEM, viscoelastic testing may ultimately become standard of care for the assessment of coagulopathy. Currently, however, the majority of basic science and clinical coagulopathy research still utilizes conventional laboratory testing with the optional addition of viscoelastic testing.

Other less utilized tests that have been used to assess for coagulopathies include platelet function testing using platelet aggregometry as well as histological and plasma-based detection of biomarkers associated with endothelial damage [50, 54, 55]. Platelet aggregometry assesses changes in electrical impedance following platelet adherence to metal electrodes to monitor for specific platelet dysfunctions based on the reagents used. Histopathology assessing for destruction of the endothelial glycocalyx has been described as this is a known process that aides in worsening coagulopathy. Other indicators of endothelial damage and ongoing coagulation derangements include (but not limited to) biomarkers such as syndecan-1, histone-complexed DNA fragments, brain-derived microparticles, activated protein C, individual factor levels, and fibrinogen.

Experimental Design

Equally as important as the initial background research to assure the proper animal for the study is the actual experimental design itself. While the major considerations include the utilization of proper animal model and the creation of the coagulopathy, other important considerations include the ethics associated with the research plan, the effects of anesthesia on the animal's physiology, the appropriate use on control groups, and the logistical requirements for each study.

Prior to instituting any animal research, a series of ethical considerations need to be addressed and adhered to in order to assure appropriate use and handling of the study subjects. In animal models that undergo traumatic injuries, as well as survival injuries, this becomes increasingly apparent as these scenarios tend to draw increased attention from internal and external regulatory committees. The *Guide for the Care and Use of Laboratory Animals* published by the Institute for Laboratory Animal Research is a five-chapter guide which serves toward initiating animal research and the underlying principles that need to be addressed. Institutional Animal Care and Use Committee (IACUC) approval is further needed per each institution's local policies. IACUC approval typically consists of a multispecialty board that reviews the scientific merit, experimental design, proposed statistical analysis, and veterinary concerns of any potential animal-based research study prior to granting approval. This regulatory board acts to assure clinically appropriate research is being conducted while maintaining the proper safety and ethical considerations surrounding animal use. A major component to this approval process is the adherence to the concept of the "three Rs": reduction, refinement, and replacement. Reduction aims to minimize the number of animals to the smallest sample size necessary to maintain adequate statistical power as determined by an a priori power analysis. Refinement seeks to address modifications to the animal husbandry or experimental procedures to minimize pain and distress while optimizing animal well-being. Finally, replacement refers to the assurance that other inanimate models or systems cannot be substituted within the study [56]. Just as the IACUC board is often comprised of interdisciplinary members, it is crucial to assure the research team is also composed of members from the medical, scientific, statistic, and veterinary realms to assure all ethical considerations, logistical concerns, and experimental factors are accounted for.

When writing an experimental design, a commonly overlooked component pertains to the effects of the anesthesia on the chosen animal model. Similar to human injury, a traumatic injury in animals results in an activation of the sympathetic nervous system resulting in the release of epinephrine and norepinephrine. This "fight or flight" response is responsible for changes in the hemodynamic patterns demon-

strated following trauma. This normal surge of catecholamines released into the bloodstream from the activation of the sympathoadrenal axis further results in the activation of platelets and endothelial release of clotting factors to promote clot formation as the body attempts to combat any ongoing blood loss. Continued activation of this physiologic response creates ongoing endothelial damage, microthrombi, and hyperfibrinolysis and can lead to refractory shock [6, 57]. Efforts aimed at thwarting the over-activation of the sympathoadrenal axis have demonstrated the potential to lessen the degree of coagulopathy due to decreased amounts of induced endothelial damage, platelet and clotting factor activation, and microthrombi formation. Volatile anesthetic gasses decrease the overall sympathetic tone and are therefore felt to display protective properties against the over-activation of the sympathoadrenal axis which has been demonstrated in previous animal models via improved glycocalyx stabilization and decreased platelet activation [58, 59]. Despite this, these gasses also have hemodynamic effects such as vasodilatation and resultant hypotension which need to be taken into account [60].

The procedures and experimental techniques performed by the research team also need to be scrutinized. Many coagulopathy studies require various injuries or surgical procedures which result in prolonged periods of open exposure and breakdown of the body's thermoregulatory system. Extended periods of an open abdomen, open chest, or extensive soft tissue injury expose the animal to rapid amounts of heat loss and significantly place the subject at risk for increased coagulopathy secondary to hypothermia. Furthermore, hemodynamic effects and the accompanying decrease in metabolic need experienced while under general anesthesia have been demonstrated to decrease core temperature by roughly 3 degrees Celsius intraoperatively [61]. With hypothermia being a key tenet in allowing for the propagation of coagulopathy, this concept should be kept in mind during model development as an unaccounted hypothermia may result in an experimentally refractory coagulopathy depending on the accompanying injury patterns. As such, methods to combat intraoperative hypothermia such as a heated surgical table, warming blankets, and warmed fluid administration may warrant consideration when accounting for all the variables that one may encounter.

Finally, when designing an experimental protocol surrounding a clinical question, all efforts should be maintained to create appropriate control and sham groups for comparison. Ideally, these groups will contain the same variables as the experimental models in order to represent a direct reflection of the intervention performed. This, however, is not always possible depending on the question being studied. When feasible, most well-designed studies utilize both a control and sham group. Control groups typically are representative of a healthy population who experiences the procedures performed however do not undergo injury or treatment. This cohort is used to represent the effects of the procedures within the model under ideal circumstances. Sham cohorts, by contrast, undergo the injury and surgical procedures without being given the intervention in question. This subsequently represents the effects of the injury within the worst-case scenario. Vehicle controls such as normal saline should be given to the sham cohorts in the precise volumes received in the experimental animal in order to account for all variables. Although the complex interplay of the multiple body systems involved during traumatic injury does not allow for a de facto direct comparison, through contrasting the sham and control groups one can extrapolate and begin to obtain an understanding of the physiologic effects of the model's specific injury patterns by assessing the key differences between the groups. Furthermore, it remains important that when comparing these two groups there are measurable differences in coagulopathy. If there are no major differences noted, then the model and associated methods for inducing coagulopathy need to be readdressed. Typically, this requires a delicate balance between increasing the injury severity while still assuring that the study goals and endpoints can be met. It is important to note that although a severe injury pattern may result in reproducible, severe coagulopathy,

repeated early demise of the study subjects can nullify an experiment via the inability to adequately obtain sufficient data.

Experimental Models

To optimize models for trauma-induced coagulopathy, it is important to have a keen understanding of the underlying mechanisms driving the pathophysiologic response. In multisystem complex polytrauma, a multitude of variables often contribute toward the coagulopathy including the ongoing hemorrhage with associated malperfusion, sepsis secondary to contamination, complex soft tissue and organ damage, and physiologic dysregulation resulting in hypothermia and acidosis. Combining all these variables creates a challenging model that would prove difficult to reliably replicate due to complexity. Therefore, researchers often will pick and choose the desired pathology to replicate in order to best address the research question. Our institutional experience largely focuses on large animal porcine models for which we have had repeated successes in inducing a trauma-induced coagulopathy through both controlled and noncontrolled hemorrhage, as well as inducing a large-scale systemic inflammatory response through aortic occlusion and then restoration of aortic blood flow to initiate the ischemia-reperfusion phenomenon [53, 62, 63]. Although the factors and underlying mechanisms that drive trauma-induced coagulopathy are complex and still not completely understood at the basic science level, the following models described within the literature result in a physiologic response that encourages coagulopathy through the promotion of metabolic acidosis, hypothermia, dysregulated immune response, and significant oxygen debt.

Isolated hemorrhage models can bring forth the desired coagulopathy in a relatively quick fashion with hypocoagulable states seen as early as 20 minutes after hemorrhage [44]. Through using a controlled hemorrhage versus an uncontrolled model, this technique works through decreasing perfusion to tissues creating hypoperfusion and end-organ damage. This ultimately releases various cytokines aiding in a systemic inflammatory state which helps to induce coagulopathy [64]. Controlled hemorrhage models are typically created utilizing serial venous drainage techniques via a large caliber central line. In our porcine experience, we have preferentially utilized a 6-9Fr catheter placed within the inferior vena cava or the left external jugular vein. In rodent models, researchers frequently cannulate the iliac vessels during controlled hemorrhage with catheters proportional to the vessel caliber being accessed. Numerous controlled hemorrhage techniques have been described, and when the hemorrhage techniques are not crucial to the research question, approaches tend to be largely dictated by institutional and local IACUC committee preference. Our institution will calculate the precise volume of blood to be withdrawn based on estimates of the animal's total blood volume and the targeted percent of hemorrhage. Blood is then withdrawn into citrated blood bags as rapidly as possible while maintaining a target mean arterial pressure. The majority of techniques described within the literature mainly revolve around the concepts of fixed-volume versus a fixed-pressure hemorrhage [65]. Our lab traditionally has utilized one type of fixed-volume approach as previously described, while other approaches utilize a constant hemorrhage rate until a desired volume is removed despite vital sign changes. A fixed-pressure approach focuses on maintaining a targeted mean arterial pressure for a certain period despite the amount of blood removed. This approach can be beneficial when assuring an animal is in a hypotensive state however will frequently result in wide variations in hemorrhage amounts between subjects due to the unpredictable, varying physiologic robustness of the animals. Noncontrolled hemorrhage models work in a similar fashion by inducing end-organ hypoperfusion and have the added benefit of the created tissue injury to release tissue factor and increase the inflammatory response. As one can speculate, these models can quickly induce death secondary to exsanguination if the injury pattern is too lethal or if hemorrhage is prolonged without resuscitation. Validated approaches to a noncontrolled hemorrhage include solid organ injury

often via a standardized splenic or liver injury, iliofemoral or other large vessel transection, and tail or other external appendage amputation [65]. While each approach offers advantages and disadvantages, a successful hemorrhage using validated methods should effectively aid in creating a coagulopathic model.

The induction of sepsis has been another well-studied technique which can initiate coagulopathy despite some contradictory data. Previous studies have demonstrated a hypocoagulable state via increased clot formation time with a decreased formation rate and speed; however, similar experimental designs have also yielded opposite findings more suggestive of a hypercoagulable state [38, 66]. Moreover, the techniques outlined within these sepsis models do bring forth criticism from skeptics due to the varying differences in the initiating trigger, as well as concern over direct clinical applicability during the early trauma resuscitation as trauma-induced coagulopathy can frequently be seen well before clinical sepsis sets in. Although these models have their limitations, these described methods have been shown to induce coagulopathy and have proven beneficial in developing a deeper understanding of various coagulopathic states. The administration of the endotoxin lipopolysaccharide (LPS) in murine models has been utilized to create acute states of inflammatory cytokines. These acute inflammatory states are brought on by doses of LPS, generally 1–25 mg/kg, much larger than required within the human populations. This technique is directly contrasted with the direct infusion of *Escherichia coli* to induce a systemic cytokine release which under the right conditions can closely mimic a disseminated intravascular coagulation picture. Just as with LPS, large quantities of bacteria are often infused which overwhelm the animal's immune system; thus, a major drawback in this model can be a low survival rate. Cecal ligation and puncture is another technique that is felt to be most representational of human sepsis and is considered the gold standard for sepsis research in animals. This technique involves ligation of the cecum distal to the ileocecal valve and perforation via needle to allow fecal contents to leak into the intraperitoneal space resulting in polymicrobial bacterial peritonitis. During this approach the severity of disease can be controlled through altering the ligation and puncture methods. Thus when attempting to mimic early trauma-induced coagulopathy, this technique allows for clinical translation to the commonly experienced hollow viscus injuries [67–69].

Isolated tissue trauma has been described with varying results and in our experience will typically result in either no measurable coagulopathy or a mild coagulopathic state that does not mimic trauma-induced coagulopathy. While one reported study demonstrated the onset of a hypocoagulable state through decreased factor V and antithrombin III following femoral nailing, isolated injury has not been reliably able to demonstrate prolongation in coagulation assays fully representational of trauma-induced coagulopathy [70]. Instead, tissue trauma causing the release of inflammatory cytokines and tissue factor is often repeated to create a polytrauma injury pattern and/or incorporated as an adjunct to other models. The intravenous infusion of tissue factor to simulate tissue trauma has also been assessed which interestingly resulted in demonstration of hyperfibrinolysis, reduced platelet function, and prolongations of coagulation assays despite there being no physical injury present [45].

Metabolic acidosis and hypothermia are two components from the "lethal triad of trauma" that are often targeted to promote a coagulopathic state [71, 72]. Creating a severe metabolic acidosis similar to that seen in a human patient in hemorrhagic shock can be extremely difficult with an animal mode, even with performing large volume hemorrhage. This is due to the relatively robust adaptive response of many animals to isolated controlled hemorrhage while under general anesthesia. This is particularly problematic in swine, who have a baseline metabolic alkalosis and a remarkably robust acid-buffering capacity that maintains pH in a relatively normal range even with 40–50% blood volume loss. Because of this difficulty, previous researchers have utilized alternative methods to induce a significant acidosis that may have little comparability to a true post-traumatic acidosis. This has included the

administration of excess intravenous acids, such as infusing hydrochloric acid until a desired pH is reached [72]. An alternative technique to promote severe and sustained metabolic acidosis in swine that we feel is much more representative of severe injury involves hemorrhage coupled with aortic occlusion and then restoration of flow to produce a significant ischemia-reperfusion injury [53]. Traditionally our lab has focused on using an aortic cross clamp to induce downstream ischemia; however, recent advancements in trauma resuscitation and the development of the resuscitative endovascular balloon occlusion of the aorta (REBOA) catheter have shifted our focus to this more minimally invasive endovascular approach to cease truncal aortic blood flow. Our experience has demonstrated that models receiving either aortic cross clamp or a supradiaphragmatic (zone 1) REBOA have accurately created sufficient ischemia-reperfusion injury resulting in acidosis and coagulopathy [62, 63, 73]. Induced hypothermia has further been associated with increased bleeding times and decreased fibrinogen availability [74–76]. As such, both passive and active cooling have been utilized in previous models to target various temperatures. The administration of cooled crystalloid further offers the advantage of aiding in the creation of a dilutional coagulopathy. Finally, as previously mentioned, general anesthesia, open surgical procedures, and large amounts of soft tissue injury frequently result in excessive heat loss which can be used advantageously for coagulopathy models.

Iatrogenic hemodilution techniques are commonly performed due to logistical ease and its ability to be standardized between subjects. This approach is often optimized when performed in combination with a controlled hemorrhage where equal or greater amounts of the shed blood volume are replaced with crystalloid or colloid solutions. In both human and animal studies, large volume crystalloid and colloid infusions have been known to cause coagulopathy. Studies have reported coagulation cascade dysfunction following large volume crystalloid administration through derangements in conventional coagulation panels, thrombin generation, fibrinogen utilization, coagulation factor consumption, endothelial glycocalyx disruption, and platelet dysfunction [77–79]. Interestingly, multiple studies assessing the clotting properties of hemodilution models have reported a decreased R-time on viscoelastic testing which remains suggestive of an accelerated initial clotting time despite there being proportionally fewer coagulation factors present [80–83]. Contrary to this, prolongation of the R-time has been demonstrated when employing a hemodilutional model using artificial colloid solution [77, 84]. Despite their differences in clot time on viscoelastic testing, both colloid and crystalloid solutions have been utilized successfully to induce a coagulopathic state [77, 84–86]. The hemodilutional approach further can be analogous to the large volumes of crystalloid resuscitation solution that patients sometimes receive during the prehospital period – a practice that is rapidly fading due to efforts to administer blood and blood products at the earliest stages possible. Furthermore, the high chloride content within normal saline solution can induce a metabolic acidosis not commonly associated with lactated Ringer's solution when administered in high volumes [87, 88]. This acidosis may further contribute to ongoing coagulopathy during model development.

Coagulopathy following isolated traumatic brain injury (TBI) has been readily demonstrated within the human population [89]. Although the underlying mechanisms for this are poorly understood, systemic coagulopathy has been demonstrated within minutes following injury through both traditional coagulation studies and viscoelastic testing [37, 50, 90]. One current theory is that brain-derived cellular microvessicles are rapidly released into the bloodstream following injury [91, 92]. Following TBI, a transient hypercoagulable state occurs in the acute period within minutes followed by a late hypocoagulopathy peaking 3–6 hours following injury [93, 94]. Furthermore, platelet dysfunction as evidenced by a decrease in adenosine diphosphate-induced platelet aggregation, endothelial glycocalyx shedding representative of endothelial damage, and hyperfibrinolysis have all been seen in TBI models [95–97]. Both isolated TBI and TBI with an associated hemorrhage have been used to

induce coagulopathies in rodent and porcine models. Rodent models typically utilize impacting devices to create local tissue injury via either transcranial methods or through a craniotomy. Porcine models, in contrast, all require a craniotomy with injury created via either fluid percussion or computer-controlled cortical impact. Other described techniques include using shock waves to mimic the effects of a blast injury on the central nervous system, penetrating ballistic-like models using a high-energy projectile, and weight drop methods where various free-falling weights are dropped onto the subjects head from above [98–101].

Conclusion

Trauma-induced coagulopathy remains a worldwide concern within the trauma community and frequently results in major morbidity and mortality. This, along with major advancements in trauma resuscitation strategies, has resulted in a major surge of research being aimed at novel methods/techniques to diagnose, mitigate, and correct the coagulopathy to restore homeostasis and achieve hemostasis. Through these efforts a multitude of models in various species have been developed, refined, and optimized in order to create reproducible, clinically translatable scenarios with rodent and porcine models encompassing a large majority of the work.

The induction of hemorrhage, sepsis, tissue trauma, hypothermia, acidosis, hemodilution, and TBI has contributed significantly to the knowledge base surrounding trauma-induced coagulopathy. Using a combination of tests to include traditional coagulation panels (prothrombin time, activated partial thromboplastin time, and international normalized ratio) and viscoelastic tests (rotational thromboelastometry and thromboelastography), as well as more specific tests assessing platelet function, individual coagulation factors, and systemic markers of endothelial damage, hypocoagulable states can be accomplished and monitored throughout the duration of the experiment. Interestingly, TBI models portray a unique phenotypic pattern which results in a hypercoagulable state during the acute post-injury period with a subsequent hypocoagulopathy of trauma multiple hours following the insult. Further study is warranted to explore this phenomenon as this phenotype has not been reliably demonstrated within the other models. Some have postulated that during the early post-injury period there is an initial surge of coagulation system activation that causes a transient hypercoagulable state in humans resulting in the rapid exhaustion of innate clotting factors leading to the hypocoagulopathies seen on presentation.

Animal models currently represent the most realistic, nonhuman studies as they allow for monitoring of the complex interconnections within the organ systems. Yet, no model is a perfect representation, and the current models continue to reveal important poorly understood concepts within trauma-induced coagulopathy. The majority of recent trials have revolved around developing new ways to reverse the coagulopathy during resuscitation. While these highly important trials address the end goal in resuscitation efforts, more energy directed toward studying post-injury/pre-resuscitation periods to improve the collective understanding around trauma-induced coagulopathy should be sought. Just as with other pathologies, developing a deeper understanding of the pathways and mechanisms of this morbid process will help to create novel therapeutics to correct the dysregulated physiologic response.

When initiating any research project, one should assure adequate injury is created to yield an appropriate physiologic response by utilizing the various techniques described within this chapter. Complex polytrauma models may more accurately reflect the injuries experienced in modern trauma; however, they also introduce multiple variables and difficulties toward elucidating the role of each insult on the animal's specific physiologic state. Although ongoing efforts within the military and civilian sectors have immensely advanced the techniques surrounding traumatic injury and damage control resuscitation, trauma-induced coagulopathy and the associated coagulopathy of trauma continue to

represent extremely difficult scenarios for clinicians. Not only does the morbidity and mortality of this pathology greatly influence patient outcomes, the massive amounts of blood products required further create a logistically burdensome challenge, especially in austere and low-resource settings. As such, it remains imperative that ongoing basic science research efforts be maintained by clinically astute researchers and physicians in order to deepen the overall understanding of trauma-induced coagulopathy and develop novel therapeutics for patient use.

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46

Applications of Viscoelastic Hemostatic Assays at the Site of Care: Considerations and Implications

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Abbreviations

CAP	College of American Pathologists
CCA	Conventional coagulation assessment
CDC	Centers for Disease Control
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare and Medicaid Services
COLA	Commission on Office Laboratory Accreditation
DOAC	Direct oral anticoagulant
FDA	Food and Drug Administration
HEMS	Helicopter Emergency Medical Service
ICU	Intensive care unit
LIS	Lab Information System
OR	Operating room
POC	Point of care
POCT	Point-of-care testing
TJC (formerly JCAHO)	The Joint Commission
VHAs	Viscoelastic hemostatic assays

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Introduction

Treatment of trauma patients requires purposeful clinical testing to ensure optimal patient care and clinical outcomes. Many of the most common tests, including conventional coagulation assessment (CCA), are sent to the central laboratory for evaluation. But an increasing number of tests are performed at the point of care [1, 2].

Point-of-care testing (POCT) is typically defined as decentralized clinical laboratory testing that is performed at or near the site of clinical care. It is often performed by operators that are not primarily trained in clinical laboratory procedures. Testing at or near the point of care has a number of benefits and challenges. Benefits include the shorter turnaround time, with earlier diagnostic insights, and the ability to respond and intervene more rapidly. It also enables more frequent measurements with direct availability of the results to the treating physician. Often, the portability of devices allows for multiple uses across different settings, i.e., emergency department (ED), operating room (OR), and surgical intensive care unit (SICU).

The challenges of POCT include a less controlled, decentralized environment with limited standardization that can lead to higher test result variability and the need to train a broader and often less experienced group of operators. Technical aspects such as maintenance, troubleshooting, and connectivity and transfer of data to

hospital IT systems present further challenges. If performed under the hospital's central lab license, issues of adequate operator training (and documentation thereof), validation, proficiency testing, and sufficient test documentation arise for users such as trauma surgeons, emergency physicians, anesthesiologists, or ED, OR, and SICU nurses who use these devices in a range of clinical settings. If point-of-care testing is performed under a separate license, the same regulatory requirements have to be adhered to and documented.

Regulatory Considerations

The US Food and Drug Administration (FDA) is responsible for the clearance or approval of all medical devices, including in vitro diagnostics, as well as overseeing their manufacture, performance, and safety. While the FDA can define intended use via their marketing authorization, including the required environment and personnel allowed to operate the device, "point of care" is itself not an FDA-defined term.

An important regulatory framework is the Clinical Laboratory Improvement Amendments (CLIA) of 1988. CLIA regulate laboratory testing and require certification of clinical laboratories by their state and the Centers for Medicare and Medicaid Services (CMS) before they can accept human samples for diagnostic testing. Three federal agencies are responsible for CLIA: the FDA, the CMS, and the Centers for Disease Control (CDC). Each agency has a unique role in assuring quality laboratory testing (Table 46.1).

Under CLIA, tests are categorized in one of two ways: waived tests (simple laboratory examinations and procedures that have an insignificant risk of an erroneous result, as defined by the CMS) and non-waived tests (further categorized to moderately complex and highly complex) [3]. POCT typically refers to waived or non-waived laboratory tests performed at remote locations by non-laboratory personnel.

CLIA accreditation as well as inspections can be performed by the CMS and other CMS-deemed organizations including the College of American Pathologists (CAP), the Commission on Office Laboratory Accreditation (COLA), and The Joint Commission (TJC, formerly JCAHO), among others.

Table 46.1 Specific roles of federal agencies responsible for the Clinical Laboratory Improvement Amendments (CLIA) [47]

Agency	Roles
FDA	Categorize tests based on complexity Review requests for waiver by application Develop rules/guidance for CLIA complexity categorization
CMS	Issue laboratory certificates Collect user fees Conduct inspections and enforce regulatory compliance Approve private accreditation organizations for performing inspections, and approve state exemptions Monitor laboratory performance on proficiency testing (PT) and approve PT programs Publish CLIA rules and regulations
CDC	Provide analysis, research, and technical assistance Develop technical standards and laboratory practice guidelines, including standards and guidelines for cytology Conduct laboratory quality improvement studies Monitor proficiency testing practices Develop and distribute professional information and educational resources Manage the Clinical Laboratory Improvement Advisory Committee

During laboratory inspections, CMS deficiencies are generally categorized as either standard level (less serious) or condition level (more serious). Commonly reported standard level deficiencies include inadequately trained personnel, improper reagent storage, expired reagents, inadequate maintenance records, and analytical measurement range verification. The most common condition level deficiencies (six out of the top ten citations) include personnel deficiencies such as unsuitable qualification requirements of the laboratory director, technical consultant, supervisors, and testing personnel [4].

As mentioned above, the focal points of POCT accreditation are training, staff competency assessments, and appropriate performance and reporting procedures. POCT can be

categorized as highly complex, even when laboratories are fully compliant, if used in a way that deviates from the manufacturer's approved labeling. Staff qualifications for highly complex tests are even more stringent. Failure to meet compliance standards can result in citations being imposed or, in the case of severe or repeated infractions, the removal of laboratory accreditation. Electronic interfacing of POCT devices with the Lab Information System (LIS) can assist program managers in maintaining compliance standards. The LIS allows centralized oversight, monitoring, and configuration of POCT devices, to aid with standardized reporting.

Viscoelastic POCT in Trauma

Viscoelastic hemostatic assays (VHAs) have long been established to assess the coagulation status of patients in a number of clinical settings, including trauma, cardiac surgery, and liver transplantation [5–14]. While most see viscoelastic tests as a useful addition to standard coagulation tests, others have concluded that viscoelastic testing upon admission to the emergency department could replace conventional coagulation tests in trauma patients altogether [15]. Goal-directed hemostatic resuscitation based on thromboelastography is reported to utilize less plasma and platelet transfusions and has also been shown to improve survival compared with a massive transfusion protocol guided by conventional coagulation assessment [16]. Another focus of VHA research is the identification of pathologies of fibrinolysis and the guidance of treatment with tranexamic acid [17–28]. A recently highlighted critical issue is the rapid detection and classification of direct oral anticoagulants (DOACs) in trauma patients [29]. While coagulation tests have traditionally been performed in the central lab, VHA technologies have opened the door for point-of-care applications.

In-Hospital POC Use In order to reduce wait times and to allow for rapid result utilization, some institutions using VHAs for trauma care have placed the devices near the point of care, e.g., in the trauma bay, OR, or SICU. In conjunction with regulatory considerations, users also have to ensure an appropriate environment, with adequate humidity and temperature and away from sources of heavy vibration, etc.

Prehospital POC Use Some investigators have explored opportunities to bring viscoelastic testing into prehospital care (experimental, investigational setting). Initially driven by military research, there is now also civilian use including testing in ambulances and helicopters [30–32]. For the use in ambulances and helicopters, the reduced vibration sensitivity of some of the newer-generation devices is critical. Another important consideration of using mobile POC testing is ensuring continuous power supply to the machines. The COMBAT trial has established the feasibility of an independent lithium battery source [33].

Overview of VHA Technologies for POCT

Given that VHA technology is described in more detail elsewhere in this book, this discussion is focused on POC aspects of the technology.

First-generation devices like the *TEG®5000 Hemostasis Analyzer* (Haemonetics) and the *ROTEM® delta* (Instrumentation Laboratory) are sometimes set up for in-hospital POC testing in a hybrid lab/POC (e.g., trauma bay, OR) approach, but more often they are still situated in the central lab. The operational complexity of the devices and the limited portability have hindered further POC use.

Next-generation devices include the TEG®6s Hemostasis Analyzer (Haemonetics), the ROTEM® sigma analyzer (Instrumentation Laboratory), and the Quantra® analyzer (HemoSonics). Of these, the *TEG®6s* Hemostasis Analyzer is currently (as of June 2020) the only portable, cartridge-based next-generation device currently cleared by the FDA in a trauma setting [34, 35]. Its reduced weight and greatly simplified use compared with other devices, paired with

its increased robustness and decreased vibration sensitivity, have enhanced its utility for in-hospital settings and beyond. In a recent study at the US Army Institute of Surgical Research, the TEG®6s Hemostasis Analyzer was evaluated during simulated ground and high-altitude aeromedical evacuation with extracorporeal life support. The authors concluded that mobile TEG®6s measurement during ground and altitude transport is feasible and provides unprecedented information to guide coagulation management. They suggested assessing the precision and accuracy of the TEG®6s Hemostasis Analyzer during transport of critically ill patients in further studies [31]. The Greater Sydney Area Helicopter Emergency Medical Service (HEMS) conducted an experiment with the TEG®6s to assess the reliability of thromboelastography in a simulated rotary wing setting. They concluded that the TEG®6s is viable technology in this setting and that it is feasible to conduct further studies using human blood in live rotary wing conditions [30]. Clinical studies with the *ROTEM® sigma* in trauma setting in the hospital are ongoing in the USA [36], and there is clinical use in trauma outside the USA, where the ROTEM® sigma is approved by regulators [37]. A recent comparison study between the ROTEM® delta and sigma showed a good correlation overall, although the validity of the methodology to define the reference ranges was later questioned and there were some differences at or around key clinical decision points [38, 39]. The *Quantra®* analyzer recently received FDA clearance (de novo pathway) for POC coagulation assessment in cardiac and major orthopedic operations based on a study in these two areas, but it has not been cleared for use in trauma (as of June 2020). Studies have shown moderate comparability with TEG® and ROTEM® analyzer technology [40–42], and further clinical trials are expected for this new device. Other experimental *optical technologies* (e.g., laser based) are currently under evaluation [43–46]. Further research is needed to assess their utility in clinical testing and specifically in a point-of-care context.

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