Practical Transfusion Medicine

SIXTH EDITION

EDITED BY
MICHAEL F. MURPHY
DAVID J. ROBERTS
MARK H. YAZER
NANCYM. DUNBAR

WILEY Blackwell

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Edited By

Michael F. Murphy, MD, FRCP, FRCPath, FFPath
Professor of Blood Transfusion Medicine
University of Oxford
Consultant Haematologist
NHS Blood and Transplant and Department of Haematology, Oxford University Hospitals
Oxford, UK

David J. Roberts, MB, ChB, DPhil, FRCPath
Professor of Haematology
University of Oxford
Consultant Haematologist
NHS Blood and Transplant and Department of Haematology, John Radcliffe Hospital
Oxford, UK

Mark H. Yazer, MD
Professor of Pathology
University of Pittsburgh
Adjunct Professor of Clinical Immunology
University of Southern Denmark
Odense, Denmark
Medical Director
RBC Serology Reference Laboratory, ITXM Centralized Transfusion Service
Associate Medical Director
ITXM Centralized Transfusion Service
Pittsburgh, PA, USA

Nancy M. Dunbar, MD
Associate Professor
Geisel School of Medicine
Department of Pathology and Laboratory Medicine
Medical Director
Blood Bank
Associate Medical Director
Transfusion Medicine Service
Dartmouth-Hitchcock Medical Center
Lebanon, NH, USA

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Contents

List of Contributors

Sandaruwani Abeysiri
The Royal Marsden NHS Foundation Trust, London, UK

Louis Alarcon
University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Jean Pierre Allain
NHS Blood and Transplant, Cambridge, UK
Division of Transfusion Medicine, Department of Haematology, University of Cambridge, Cambridge, UK

Caroline R. Alquist
Transplantation Immunology & Therapeutic Apheresis, Cincinnati, OH, USA
Hoxworth Blood Center, University of Cincinnati, Cincinnati, OH, USA

Ravishankar Rao Baikady
The Royal Marsden NHS Foundation Trust, London, UK

Imelda Bates
Liverpool School of Tropical Medicine, Liverpool, UK

Neil Blumberg
Department of Pathology and Laboratory Medicine, University of Rochester, Rochester, NY, USA
Blood Bank/Transfusion Service of Strong Memorial Hospital, Rochester, NY, USA

Franklin Bontempo
University of Pittsburgh Medical Center, Pittsburgh, PA, USA
Vitalant, Pittsburgh, PA, USA

Susan J. Brunskill
Systematic Review Initiative, NHS Blood and Transplant, Oxford, UK

Alexandra Budhai
Memorial Sloan Kettering Cancer Center, New York, NY, USA

Rebecca Cardigan
NHS Blood and Transplant, Cambridge, UK

Ronjon Chakraverty
Department of Haematology, Churchill Hospital, Oxford, UK
BRC Haematology Theme, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Akila Chandrasekar
NHS Blood and Transplant, Tissue and Eye Services, Liverpool, UK

Irina Chibisov
University of Pittsburgh Medical Center, Pittsburgh, PA, USA
Vitalant, Pittsburgh, PA, USA

Martin Shao Foong Chong
The Royal Marsden NHS Foundation Trust, London, UK

Fateha Chowdhury
NHS Blood and Transplant, London, UK
Imperial College Healthcare NHS Trust, Imperial College, London, UK

Brian R. Curtis
Platelet and Neutrophil Immunology Laboratory and Blood Research Institute, Versiti, Milwaukee, WI, USA

Robert D. Danby
Churchill Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Geoff Daniels
International Blood Group Reference Laboratory, NHS Blood and Transplant, Bristol, UK

Robertson D. Davenport
University of Michigan Health System, Ann Arbor, MI, USA

Meghan Delaney
Department of Pathology & Laboratory Medicine Division, Children's National Hospital, Washington, DC, USA
Department of Pathology & Pediatrics, The George Washington University, Washington, DC, USA

Gregory A. Denomme
Versiti Blood Research Institute and Diagnostic Laboratories, Milwaukee, WI, USA

Aggrey Dhabangi
Child Health and Development Centre, Makerere University College of Health Sciences, Kampala, Uganda

Roger Y. Dodd
American Red Cross, Jerome H. Holland Laboratory for the Biomedical Sciences, Rockville, MD, USA

Killian Donovan
Oxford University Clinical Academic Graduate School, John Radcliffe Hospital, Oxford, UK
Adult Intensive Care Unit, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Carolyn Dorée
Systematic Review Initiative, NHS Blood and Transplant, Oxford, UK

Heidi Doughty
NHS Blood and Transplant, Birmingham, UK
NIHR Surgical Reconstruction and Microbiology Research Centre, Institute of Translational Medicine, Birmingham, UK

Katharine A. Downes
University Hospitals Cleveland Medical Center and Case Western Reserve University, Cleveland, OH, USA

Steven J. Drews
Microbiology, Canadian Blood Services, Edmonton, Alberta, Canada
Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada

Walter H. (Sunny) Dzik
Department of Pathology and Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Khaled El-Ghariani
NHS Blood and Transplant, Sheffield, UK
Sheffield Teaching Hospitals NHS Trust, Sheffield, UK
University of Sheffield, Sheffield, UK

Lise J. Estcourt
NHS Blood and Transplant, John Radcliffe Hospital, Oxford, UK
Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Dean Fergusson
Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

Stephen P. Field
Irish Blood Service, National Blood Centre, Dublin, Ireland

Peter Flanagan
New Zealand Blood Service, Wellington, New Zealand

Ronan Foley
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Richard O. Francis
Columbia University Medical Center, New York, NY, USA

Steven M. Frank
Department of Anesthesiology/Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

Mark K. Fung
Department of Pathology and Laboratory Medicine, University of Vermont Medical Center, Burlington, VT, USA

Marc Germain
Héma-Québec, Quebec City, Quebec, Canada

Mindy Goldman
Canadian Blood Services, Ottawa, Ontario, Canada
University of Ottawa, Ottawa, Ontario, Canada

Lawrence Tim Goodnough
Departments of Pathology and Medicine, Stanford University, Stanford, CA, USA

Andreas Greinacher
Department of Immunology and Transfusion Medicine, Universitätsmedizin Greifswald, Greifswald, Germany

James Griffin
Therapeutics at NHS Blood and Transplant, Filton, Bristol, UK

Yisu Gu
Department of Haematology, Churchill Hospital, Oxford, UK
BRC Haematology Theme, Radcliffe Department of Medicine, University of Oxford, Oxford, UK
Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK

Akash Gupta
Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Sarah K. Harm
Department of Pathology and Laboratory Medicine, University of Vermont Medical Center, Burlington, VT, USA

Joanna M. Heal
Department of Pathology and Laboratory Medicine, University of Rochester, Rochester, NY, USA
Blood Bank/Transfusion Service of Strong Memorial Hospital, Rochester, NY, USA

Paul C. Hébert
Department of Medicine, Centre Hospitalier de L'Université de Montréal, Centre Recherche du Centre Hospitalier de Montréal, Montréal, Québec, Canada

Nancy M. Heddle
Department of Medicine, McMaster University, Hamilton, Ontario, Canada
Canadian Blood Services, Hamilton, Ontario, Canada

Nadia B. Hensley
Department of Anesthesiology/Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

Aaron S. Hess
Departments of Anesthesiology and Pathology and Laboratory Medicine, University of Wisconsin, Madision, WI, USA

John R. Hess
Departments of Laboratory Medicine and Pathology and Hematology, University of Washington, Seattle, WA, USA

Marie A. Hollenhorst
Departments of Pathology and Medicine, Stanford University, Stanford, CA, USA

Rachael Hough
University College London Hospital's NHS Foundation Trust, London, UK

Cassandra D. Josephson
Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA, USA
Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA
Department of Pathology and Laboratory Medicine, Center for Transfusion and Cellular Therapies, Emory University School of Medicine, Atlanta, GA, USA

Seema Kacker
The Johns Hopkins University School of Medicine and Bloomberg School of Public Health, Baltimore, MD, USA

Rick Kapur
Sanquin Research, Amsterdam, The Netherlands
Landsteiner Laboratory, Amsterdam UMC-AMC, Amsterdam, The Netherlands

Louis M. Katz
ImpactLife Blood services, Davenport, IA, USA
Infectious Diseases, Carver College of Medicine, University of Iowa Healthcare, Iowa City, IA, USA

Richard M. Kaufman
Brigham and Women's Hospital, Boston, MA, USA

Robert B. Klanderman
Department of Intensive Care and Department of Anesthesiology, Amsterdam UMC–AMC, University of Amsterdam, Amsterdam, The Netherlands

Heather Leary
Department of Haematology, Churchill Hospital, Oxford, UK

Christine M. Leeper
University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Marissa Li
Cerus Corporation, Concord, CA, USA

Na Li
Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

Yulia Lin
Precision Diagnostics and Therapeutics Program, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

Richard Lomas
NHS Blood and Transplant, Tissue and Eye Services, Liverpool, UK

Edwin J. Massey
Consultant Haematologist, Velindre University NHS Trust and Cwm Taf Morgannwg University Health Board, Talbot Green, Wales, UK

Stuart McKechnie
Adult Intensive Care Unit, Oxford University Hospitals NHS Foundation Trust, Oxford, UK
Nuffield Department of Anaesthesia, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Zoe K. McQuilten
Transfusion Research Unit, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia
Department of Clinical Haematology, Monash Health, Melbourne, Australia

Jay E. Menitove
Community Blood Center of Greater Kansas City, Kansas City, MO, USA (retired)
Pathology and Laboratory Medicine, Kansas University Medical Center, Kansas City, KS, USA

Siraj A. Misbah
Oxford University Hospitals, University of Oxford, Oxford, UK

Celina Montemayor
Centre for Innovation, Canadian Blood Services, Toronto, Ontario, Canada

Katrina M. Morgan
University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Michael F. Murphy
NHS Blood and Transplant, John Radcliffe Hospital, Oxford, UK
Department of Haematology, Oxford University Hospitals, Oxford, UK
Radcliffe Department of Medicine, University of Oxford, Oxford, UK

William G. Murphy
Irish Blood Transfusion Service (retired)
University College Dublin, Dublin, Republic of Ireland

Matthew D. Neal
University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Paul M. Ness
Transfusion Medicine Division, Pathology and Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD, USA

Lirong Qu
Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA
Vitalant, Pittsburgh, PA, USA

Richard Rackham
NHS Blood and Transplant, London, UK

Alex Rampotas
Department of Haematology, Churchill Hospital, Oxford, UK

Sandra Ramírez-Arcos
Medical Affairs and Innovation, Canadian Blood Services, Ottawa, Ontario, Canada
Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada

Majed A. Refaai
Department of Pathology and Laboratory Medicine, University of Rochester, Rochester, NY, USA
Blood Bank/Transfusion Service of Strong Memorial Hospital, Rochester, NY, USA

Toby Richards
University of Western Australia, Perth, Australia

David J. Roberts
BRC Haematology Theme, Radcliffe Department of Medicine, University of Oxford, Oxford, UK
NHS Blood and Transplant, Oxford, UK
Department of Haematology, John Radcliffe Hospital, Oxford, UK

Martin Rooms
The Royal Marsden NHS Foundation Trust, London, UK

Paul Rooney
NHS Blood and Transplant, Tissue and Eye Services, Liverpool, UK

Eric Salazar
Department of Pathology & Genomic Medicine, Houston Methodist Hospital, Houston, TX, USA

Richard Salisbury
Churchill Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Jansen Seheult
University of Pittsburgh Medical Center, Pittsburgh, PA, USA
Vitalant, Pittsburgh, PA, USA

Akshay Shah
Radcliffe Department of Medicine, University of Oxford, Oxford, UK
Adult Intensive Care Unit, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Beth H. Shaz
Duke University, Durham, NC, USA

Patricia A. Shi
Clinical Services, New York Blood Center, New York, NY, USA
Division of Hematology, Department of Medicine, Albert Einstein College of Medicine, New York, NY, USA
Division of Hematology-Oncology, Department of Medicine, Northwell Health, New Hyde Park, New York, NY, USA

Andrew W. Shih
Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

Edward L. Snyder
Department of Laboratory Medicine, Transfusion Medicine Service, Yale University, School of Medicine/Yale–New Haven Hospital, New Haven, CT, USA

Lynn G. Stansbury
Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA

Simon J. Stanworth
Radcliffe Department of Medicine, University of Oxford, Oxford, UK
Transfusion Medicine, NHS Blood and Transplant, Oxford, UK
Department of Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Sean Stowell
Joint Program in Transfusion Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

H. Cliff Sullivan
HLA Laboratory, Director of Cellular Therapy, Center for Transfusion and Cellular Therapies, Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, USA

Connor Sweeney
Department of Haematology, Churchill Hospital, Oxford, UK
BRC Haematology Theme, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Zbigniew (Ziggy) M. Szczepiorkowski
Transfusion Medicine Service, Cellular Therapy Center, Dartmouth-Hitchcock Medical Center and Geisel School of Medicine at Dartmouth, Hanover, NH, USA
Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Yvette C. Tanhehco
Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY, USA

Suzanne R. Thibodeaux
Division of Laboratory and Genomic Medicine, Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA

Stephen Thomas
NHS Blood and Transplant, London, UK

Pierre Tiberghien
Établissement Français du Sang, La Plaine Saint-Denis, and Inserm UMR 1098 RIGHT, Université de Franche-Comté, Établissement Français du Sang, Besançon, France

Alan T. Tinmouth
Division of Hematology, Department of Medicine, Ottawa Hospital, Ottawa, Ontario, Canada
Ottawa Hospital Center for Transfusion Research, Clinical Epidemiology Program, Ottawa Health Research Institute, Ottawa, Ontario, Canada

Aaron A. R. Tobian
The Johns Hopkins University School of Medicine and Bloomberg School of Public Health, Baltimore, MD, USA

Christopher A. Tormey
Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA

Darrell J. Triulzi
Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA
Vitalant, Pittsburgh, PA, USA

Ralph Vassallo
Vitalant, Scottsdale, AZ, USA

Alexander P.J. Vlaar
Department of Intensive Care, Amsterdam UMC–AMC, University of Amsterdam, Amsterdam, The Netherlands

Theodore E. Warkentin
Department of Pathology and Molecular Medicine and Department of Medicine, Michael G. DeGroote School of Medicine, McMaster University, Hamilton, Ontario, Canada
McMaster Centre for Transfusion Research, Hamilton, Ontario, Canada
Transfusion Medicine, Hamilton Regional Laboratory Medicine Program and Service of Benign Hematology, Hamilton Health Sciences, Hamilton General Hospital, Hamilton, Ontario, Canada

Jonathan H. Waters
Departments of Anesthesiology & Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

Kathryn E. Webert
Canadian Blood Services, and Department of Medicine and Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Barbee I. Whitaker
Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA

Julia Wolf
NHS Blood and Transplant, North Bristol Park, Filton, Bristol, UK

Erica M. Wood
Transfusion Research Unit, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia
Department of Clinical Haematology, Monash Health, Melbourne, Australia

Mark H. Yazer
Professor of Pathology, University of Pittsburgh, PA, USA
Visiting Professor in Pathology, Tel Aviv University, Israel
Adjunct Professor of Clinical Immunology, University of Southern Denmark, Odense, Denmark
Associate Medical Director, Centralized Transfusion Service, Pittsburgh, PA, USA

Marianne E. Yee
Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA, USA
Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA

Preface to the Sixth Edition

The pace of change in transfusion medicine is relentless, with new scientific and technological developments and continuing efforts to improve transfusion practice. This sixth edition has been updated significantly to reflect the rapid changes in transfusion medicine since the fifth edition was published in 2017. The interval between the fifth and sixth editions has been extended by one year to allow colleagues to focus without distraction on the many challenges posed by the COVID-19 pandemic.

The primary purpose of this edition remains the same as the first, namely to provide a comprehensive guide to transfusion medicine. The book aims to include information in more depth than is contained within handbooks of transfusion medicine, and yet to present that information in a more concise and approachable manner than is seen in large, standard reference texts. The feedback we have received not only from formal and informal reviews but also from colleagues is that these objectives continue to be achieved, and that the book benefits from a consistent style and format for its chapters. We have again striven to maintain this standard in the sixth edition and to provide a text that will be useful to clinical and scientific staff, both established practitioners and trainees, who are involved in transfusion medicine and require an accessible text.

The book is divided into seven sections, which systematically take the reader through the principles of transfusion medicine, the complications of transfusion, practice in blood centres and hospitals, clinical transfusion practice, a new section on patient blood management, cellular and tissue therapy and organ transplantation and the development of the evidence base for transfusion. The main changes from the fifth edition are a new chapter on transfusion-associated circulatory overload by Alexander Vlaar to underline the condition's importance as a complication of transfusion, and a reconfiguration of the section on clinical transfusion practice to consider the transfusion management of medical, surgical and haematology patients with and without bleeding. The number of chapters has therefore been increased from 49 to 51. The first and final chapters on the recent evolution of transfusion medicine and scanning the future of transfusion medicine have always generated much interest in previous editions, and we are very grateful that Sunny Dzik, Ed Snyder, Paul Ness and Jay Menitove have provided excellent updates of their respective reviews for this edition.

We continue to develop the content and to refresh the style of the book, and are very pleased to welcome Nancy Dunbar as co-editor. The authorship has also become more international with each successive edition to provide a broad perspective. We are very grateful to the colleagues who have contributed to this book especially at this challenging time. Once again, we acknowledge the enormous support we have received from our publishers, and particularly Mandy Collison.

1 Introduction: Two Centuries of Progress in Transfusion Medicine

Walter H. (Sunny) Dzik[1] *and Michael F. Murphy*[2]

[1] *Department of Pathology and Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
[2] *Radcliffe Department of Medicine, University of Oxford; NHS Blood and Transplant, John Radcliffe Hospital; Department of Haematology, Oxford University Hospitals, Oxford, UK*

'States of the body really requiring the infusion of blood into the veins are probably rare; yet we sometimes meet with cases in which the patient must die unless such operation can be performed.' So begins James Blundell's 'Observations on transfusion of blood', published in *The Lancet*, marking the origins of transfusion medicine as a clinical discipline [1]. Blundell (Figure 1.1) was a prominent London obstetrician who witnessed peripartum haemorrhage and whose interest in transfusion had begun as early as 1817 during his medical education in Edinburgh. He established that transfusions should not be conducted across species barriers and noted that resuscitation from haemorrhage could be achieved using a volume of transfusion that was smaller than the estimated blood loss. Despite life-saving results in some patients, clinical experience with transfusion was restricted by lack of understanding of ABO blood groups – a barrier that would not be resolved for another century.

The Nobel Prize–winning work of Karl Landsteiner (Figure 1.2) established the primacy of ABO blood group compatibility and set the stage for safer transfusion practice. Twentieth-century transfusion was advanced by the leadership of many physicians, scientists and technologists and repeatedly incorporated new diagnostics (monoclonal antibodies, genomics) and new therapeutics (plasma fractionation, apheresis and recombinant proteins) to improve patient care.

Today, the field of transfusion medicine is composed of a diverse range of disciplines including the provision of a safe blood supply; the fields of haemostasis, immunology, transplantation and cellular engineering; apheresis technology; treatment using recombinant and plasma-derived plasma proteins; and the daily use of blood components in clinical medicine (Figure 1.3). Without transfusion resources, very little of modern surgery and medicine could be accomplished.

For decades, the challenge of transmitting new information in transfusion fell to Dr Patrick Mollison (Figure 1.4), whose textbook became the standard of its era. Mollison highlighted the importance of both laboratory practice (immunohaematology, haemostasis, complement biology) and clinical medicine in our field. *Practical Transfusion Medicine*, here in its sixth edition, seeks to build on that tradition and to give readers the foundation knowledge required to contribute both academically and clinically to our discipline. For readers about to enjoy the content of this book, the following provides a sampling of the topics presented within the text by leading experts in our field.

Practical Transfusion Medicine, Sixth Edition. Edited by Michael F. Murphy, David J. Roberts, Mark H. Yazer and Nancy M. Dunbar.

Figure 1.1 James Blundell. *Source:* J. Cochran / The National Portrait Gallery, Volume II, published c.1820 (litho) / Public domain..

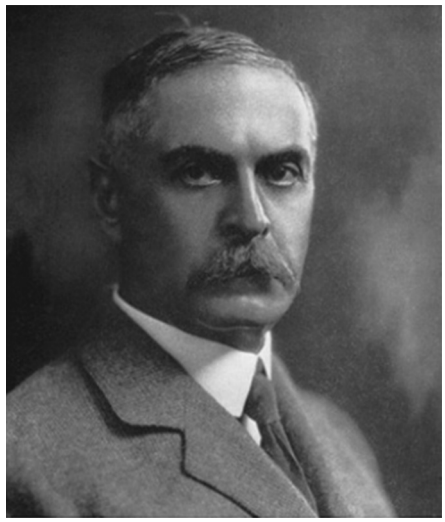

Figure 1.2 Karl Landsteiner. *Source:* VladiMens / Wikimedia Commons / Public domain..

Blood Donation Worldwide

Each year, approximately 100 million blood donations are made worldwide (Figure 1.5). A safe and adequate blood supply is now an essential infrastructure requirement of any modern national healthcare system. The

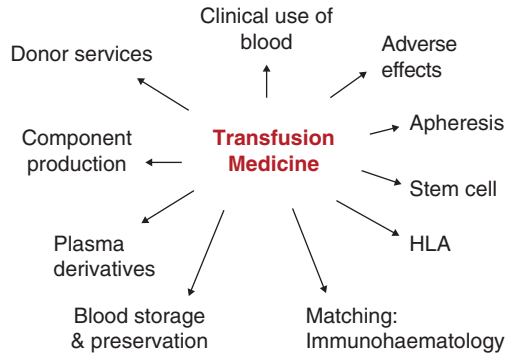

Figure 1.3 The range of transfusion medicine.

Figure 1.4 Patrick Mollison. *Source:* Garratty, Transfusion 2012;**52**:684–85. Reproduced with permission of John Wiley & Sons.

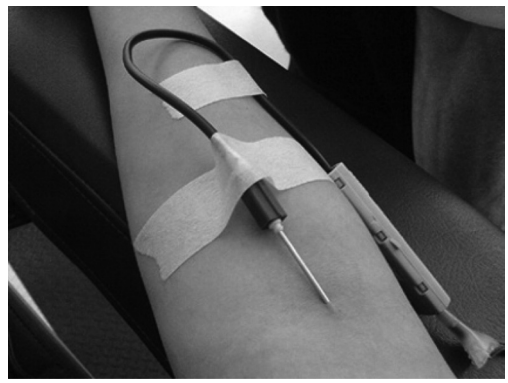

Figure 1.5 Blood donation.

recruitment and retention of healthy blood donors are vital activities of the field and the challenges and responsibilities faced by stewards of the blood supply were highlighted during the first year of the global COVID-19 pandemic, when routine blood collections were placed under great strain and when blood collecting agencies took on the added responsibility of collecting convalescent plasma to treat COVID-19 infection. While the economically advantaged nations of the world have established all-volunteer donor programmes with great success, data from the World Health Organization presented in Chapter 27 document that blood donation rates per capita in many low-income nations are insufficient to meet their needs. More research and investment are required so that all regions of the world can rely upon an adequate supply of safe blood.

Changing Landscape of Transfusion Risks

During the final two decades of the twentieth century, intense focus on screening blood donations for infectious diseases led to substantial progress in blood safety and a significant reduction in the risk of transfusion-transmitted diseases (Figure 1.6). Chapters 17–19 present an authoritative summary of this success. We currently enjoy a grace period when the risk of transfusion-transmitted infections is at an all-time low. One of the great successes of recent decades was the identification and screening of donors for hepatitis – a landmark effort that resulted in another Nobel Prize in our field, this time awarded to Harvey Alter (Figure 1.7) of the US National Institutes of Health (NIH) [2].

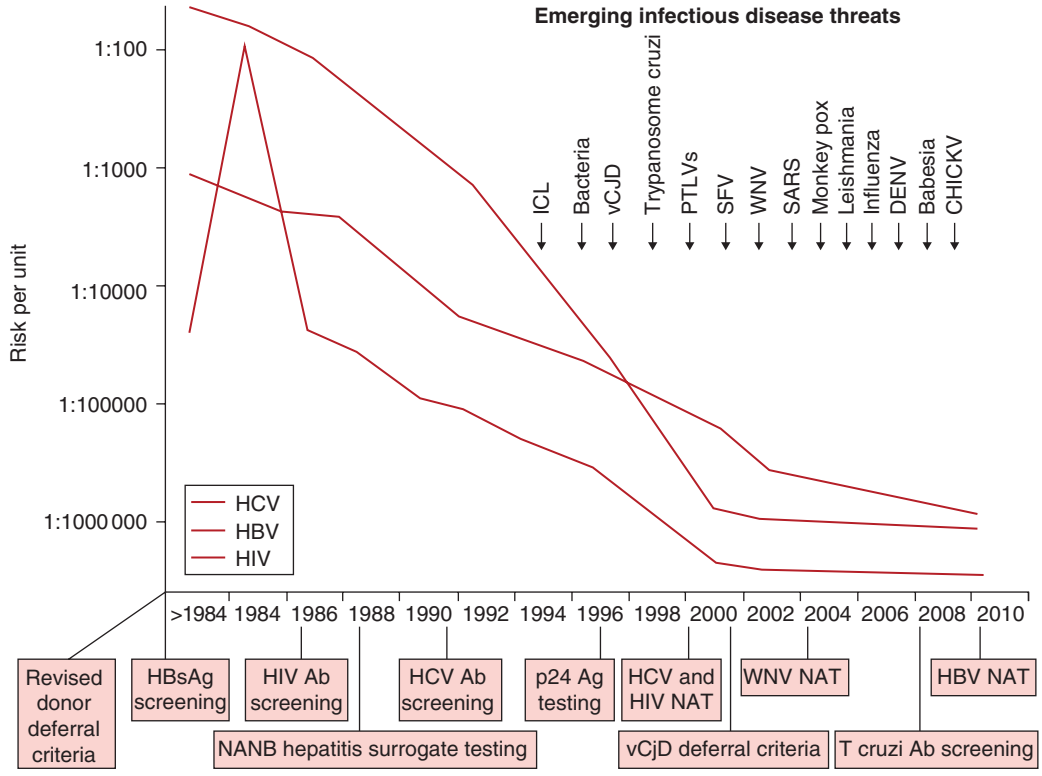

Figure 1.6 Risks of transfusion-transmitted infections over time. Ab, antibody; Ag, antigen; CHICKV, chikungunya virus; DENV, dengue virus; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; ICL, idiopathic CD4-positive T-lymphocytopenia; NANB, non-A non-B hepatitis; NAT, nucleic acid testing; PTLVs, primate T-lymphotropic viruses; SARS, severe acute respiratory syndrome; SFV, Semliki Forest virus; T cruzi, *Trypanosoma cruzi*; vCJD, variant Creutzfeldt–Jakob disease; WNV, West Nile virus.

Despite this achievement, progressive encroachment of humans upon the animal kingdom is expected to result in the emergence of new infections that cross species barriers. Haemovigilance, robust screening technologies and chemical pathogen inactivation are all being applied to address this concern and are reviewed within the text. With the advent of the twenty-first century, the landscape of transfusion risk shifted its emphasis towards non-infectious hazards (Figure 1.8). Recent years have focused on improved understanding and prevention of transfusion-related acute lung injury, a topic covered in detail in Chapter 11. More recently, we have learned that circulatory overload from excessive transfusion is far more common than previously recognised, as described in Chapter 10. Yet Blundell himself specifically warned of it in his first description of transfusion: 'to observe with attention the

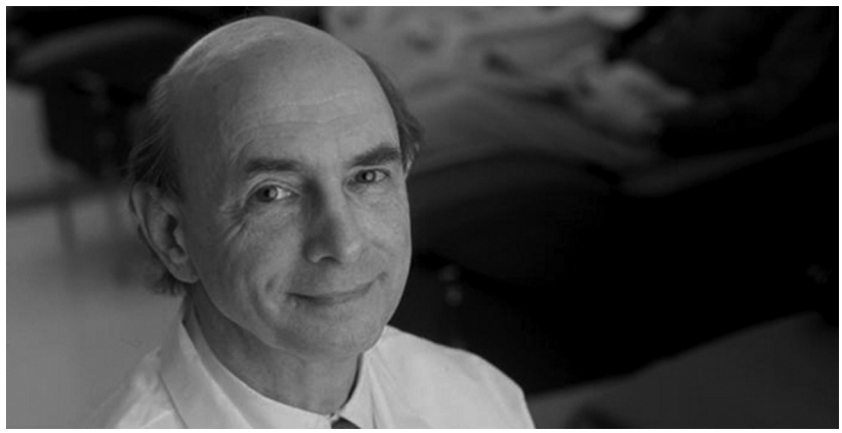

Figure 1.7 Harvey Alter. *Source:* Clinicalcenter.nih.gov

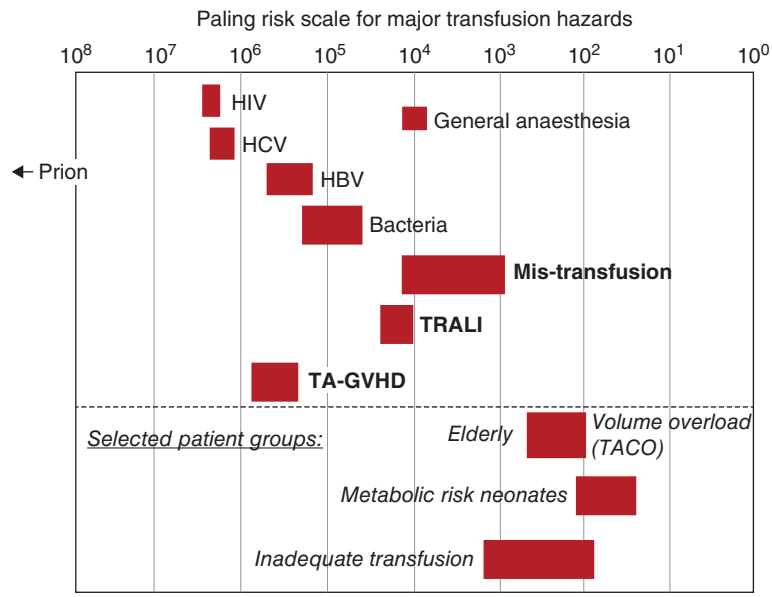

Figure 1.8 Paling scale of transfusion risk. HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; TACO, transfusion-associated circulatory overload; TA-GVHD, transfusion-associated graft-versus-host disease; TRALI, transfusion-related acute lung injury.

countenance of the patient, and to guard . . . against an overcharge of the heart' [1]. In addition, haemolytic reactions remain a serious hazard of transfusion. It is quite surprising that despite unimagined advances in internet connectivity, most nations still do not have a system for sharing patient blood group results or antibody profiles between hospitals, and are thereby failing to share information that would prevent acute and delayed reactions. Much can still be done to further reduce non-infectious hazards of transfusion. Readers will find that Chapters 7–19 provide state-of-the-art summaries of our current understanding regarding the full range of adverse effects and complications of transfusion.

Immunohaematology

Knowledge of the location and functional role of red cell surface proteins that display blood group epitopes has brought order out of what was once a chaotic assembly of information in blood group serology (Figure 1.9). Readers will enjoy an up-to-date treatment of this topic in Chapters 2–6.

Today, red cell genomics has become a practical clinical tool and DNA diagnostics in immunohaematology extends far beyond the reach of erythrocyte blood groups. Genotyping has always been the preferred method for defining members of the human platelet antigen system and is well established for HLA genes in the field of histocompatibility (Figure 1.10). The clinical practice of transfusion medicine is now supported by DNA diagnostics targeting a wide range of genes, including not only the increased use of DNA sequences that encode blood group antigens, but also those encoding complement proteins, haemoglobin polymorphisms and coagulation factors.

Despite advances in defining antigens, both clinical illness and blood group incompatibilities remain dominated by the patient's antibody responses. A robust form of antibody analysis and better control of the immune response remain important frontiers of our field. The ability to downregulate specific alloimmune responses would revolutionise the approach to solid organ transplantation, haemophilia complicated by inhibitors,

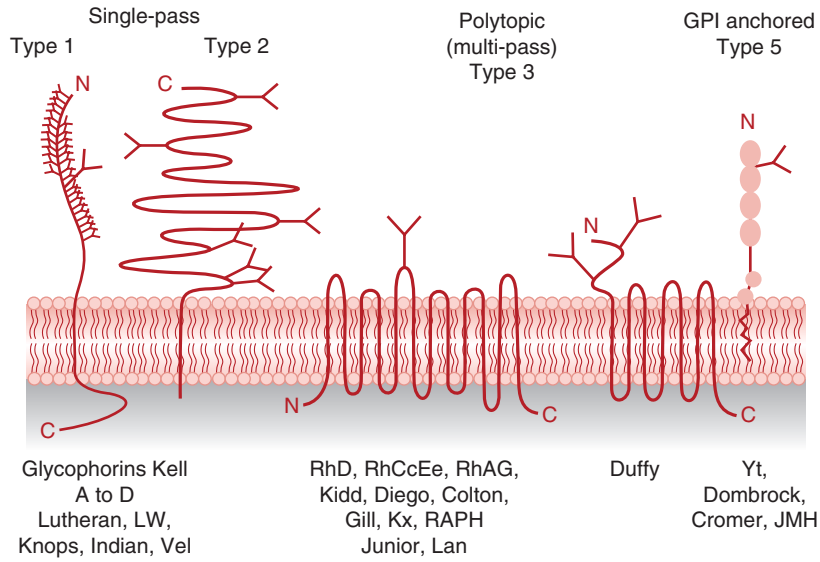

Figure 1.9 Red blood cell antigens. *Source:* Daniels G, Bromilow I. Essential Guide to Blood Groups, 3rd edn. Wiley: Chichester, 2014. Reproduced with permission of John Wiley & Sons.

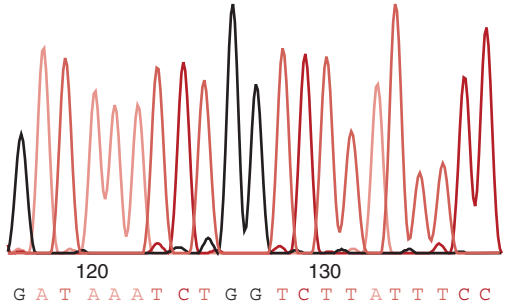

Figure 1.10 DNA sequence.

platelet refractoriness, red cell allosensitisation, haemolytic disease of the newborn and a host of other challenges that confront transfusion specialists every day.

In the meantime, we can offer patients powerful yet non-specific immune suppressants. And while the focus of many treatments is on reduction of pathological antibodies, it is increasingly clear that antibodies themselves do not injure tissues nearly as much as the complement proteins that antibodies attract. Complement is at the centre of a wide variety of disorders, including drug-mediated haemolysis or thrombocytopenia, severe alloimmune or autoimmune haemolysis, cryoglobulinaemic vasculitis, HLA antibody-mediated platelet refractoriness and organ rejection, paroxysmal nocturnal haemoglobinuria, atypical haemolytic-uremic syndrome, hereditary angioedema, glomerulonephritis and age-related macular degeneration. With the development in the future of better agents to suppress complement, it can be anticipated that the focus of treatment may shift from removal of pathological antibodies to control of their effect.

Clinical Use of Blood Components: Evolution Based on Evidence

Recent years have witnessed a growing body of evidence derived from clinical research and focused on the proper use of blood components. While such research has lagged for plasma products, progress has been made for both red cells and platelets. Ever since the landmark publication of the TRICC trial by Hebert and others [3], clinical investigators have repeatedly challenged the traditional 100 g/L haemoglobin threshold for red cell transfusion. There are now at least 13 well-designed, sufficiently powered randomised controlled trials documenting that a conservative haemoglobin threshold for red cell transfusion is as beneficial for patient outcomes as a more liberal threshold (Figure 1.11). These studies cut across a broad range of patient categories, from infants to the elderly. As a result, in hospitals worldwide, red cell use is more conservative and transfusions are now withheld in non-bleeding patients until the haemoglobin concentration falls to 70 g/L. Looking ahead, we anticipate that future clinical research will seek to further refine the indication for red cells by addressing the fact that the haemoglobin concentration is but one dimension of tissue oxygenation, and that the decision to transfuse red cells should include measures of both oxygen delivery and tissue oxygen consumption.

The last decade has also witnessed evidence-based refinements in the indication for platelet transfusion. The modern era of evidence begins with the work of Rebulla et al. [4], who documented that a platelet threshold of 10×10^9/L was equivalent to 20×10^9/L for prophylactic platelet transfusions. Further advances came with the TRAP trial [5], demonstrating that reducing the number of leucocytes (and not the number of donors) was key to preventing HLA alloimmunisation, and the PLADO trial [6], which demonstrated that the traditional dose of platelets (approximately equivalent to that found in 4–6 units of whole blood) resulted in the same outcome as transfusion of 3 units, or 12 units as judged by the proportion of days with grade 2 or higher bleeding. Finally, the TOPPS trial [7] revealed that there was little value to prophylactic platelets among clinically stable patients undergoing

Randomized Trials of RBC transfusion threshold

Author	Name	Setting	Trigger	'n'
Hebert, 1999	TRIC	Adult ICU	7 vs 9	838
Kirpalami, 2006	PINT	Infants < 1 kg	10 vs 12	457
Lacroix, 2007	---	Pediatric ICU	7 vs 9.5	637
Hajjar, 2010	TRAC	Cardiac surgery	8 vs 10	502
Cooper, 2011	CRIT	Acute MI (pilot)	8 vs 10	45
Carson, 2011	FOCUS	Hip surgery elderly	8 vs 10	2,016
Villaneuva, 2013	---	UGI bleed	7 vs 9	921
Walsh, 2013	RELIEVE	Older patients in ICU	7 vs 9	100
Robertson, 2014	---	Traumatic brain	7 vs 10	200
Holst, 2014	TRISS	Septic shock	7 vs 9	998
Murphy, 2015	----	Cardiac surgery	7.5 vs 9	2,007
Mazer, 2017	TRICS-III	Cardiac surgery	7.5 vs 9	5,243
Franz AR, 2020	ETTNO	Infants < lkg	10 vs 12	1013
Ducrocq G, 2021	REALITY	Acute MI	8 vs 10	668

Figure 1.11 Trials examining the red blood cell (RBC) transfusion threshold. ICU, intensive care unit; MI, myocardial infarction; UGI, upper gastrointestinal.

autologous bone marrow transplantation. The goal now is to conduct more research on platelet transfusion outside the context of haematological malignancy. While we still have much more to do if we are to refine the clinical use of the traditional blood components, Chapters 37–40 on patient blood management and 47–48 in the section on developing the evidence base for transfusion should give readers a solid foundation upon which to improve clinical decisions regarding transfusion.

Urgent Transfusion

Care of the haemorrhaging patient has always been an essential aspect of transfusion practice. The tragedies of war and human conflict have repeatedly stimulated research focused on urgent transfusion during haemorrhage. Demand for knowledge in this area sadly continues and is also driven by trauma occurring in civilian life, for example due to firearms and automobile injury. This is an area of changing practice patterns, including a re-examination of products such as whole blood and cold-stored platelet concentrates that were used decades ago (Figure 1.12). Readers will welcome the up-to-date focus found in Chapter 30. With the advent of increasingly complex surgery and deployment of life support systems such as extracorporeal membrane oxygenators, massive transfusion is no longer restricted to trauma. In fact, recent studies document that the majority of massive transfusion episodes are associated with surgical and medical conditions unrelated to trauma [8]. More research in these patient groups is needed.

Patients Requiring Chronic Transfusion Support

Chapters 32, 33 and 34 address the needs of patients with benign and malignant haematological disorders and those with haemoglobinopathies such as sickle cell anaemia, who often require chronic transfusion support (Figure 1.13). Patients with haemoglobinopathies, thalassaemia, myelodysplastic syndromes,

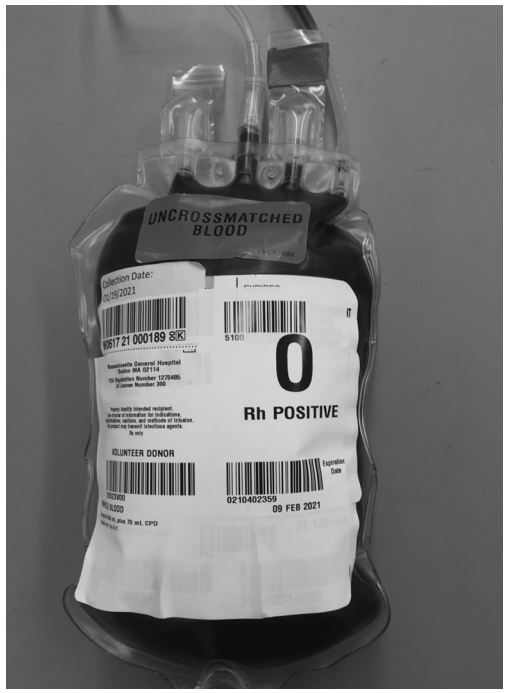

Figure 1.12 Unit of whole blood. *Source:* Sunny Dzik (Massachusetts General Hospital).

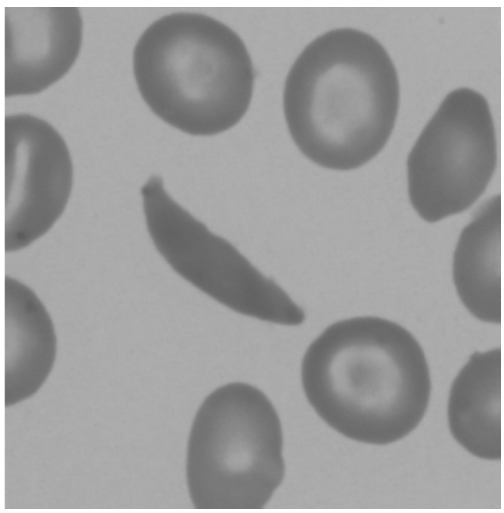

Figure 1.13 Sickle cell anaemia. *Source:* Gregory Kato / Wikimedia Commons.

aplastic anaemia, refractory anaemia, congenital and acquired haemolytic anaemia and those with chronic bleeding disorders such as hereditary haemorrhagic telangiectasia depend upon transfusion to sustain them. Worldwide, the numbers of individuals with severe uncorrectable anaemia are enormous. For these conditions, blood transfusion is seen at its raw, primal best: the sharing of blood from those in good health with those in need.

Obstetric, Neonatal and Paediatric Transfusion Medicine

Care of the low-birthweight, premature infant remains very challenging. Anaemia and thrombocytopenia result from physiology unique to these youngest of patients, as described in Chapter 35. Neonatal and paediatric transfusion medicine is filled with customary practices often based more on tradition than on evidence. We applaud those who have conducted the controlled trials that are summarised within the text, and look forward to additional clinical research designed to answer fundamental questions that confront the paediatric transfusion specialist.

Haemostasis and Transfusion

No area of transfusion medicine has seen such explosive recent innovation as the field of haemostasis. A wide range of anticoagulants is now available and the balance between anticoagulation, haemostasis and thrombophilia has become more complex. Transfusion therapy continues a long evolution from plasma replacement to the targeted use of a growing number of plasma-derived or recombinant products that influence haemostasis. Tools and treatments used in the past and then put aside, such as viscoelastic testing and antifibrinolytics, have made a strong resurgence and are finding new positions in the evaluation and treatment of bleeding. Additional haemostasis agents, which we will need to clinically master, are on the way. Chapters 28–30 address these

topics and will give readers new information on the important role of transfusion in the care of patients with disorders of haemostasis and thrombosis.

Cellular Therapies, Transplantation, Apheresis

Cellular therapy is a major growth area in transfusion medicine. The ability to mobilise haematopoietic progenitor cells, then harvest them safely in bulk numbers, process, freeze and successfully reinfuse them as a stem cell tissue transplant has completely revolutionised the field of bone marrow transplantation (Figure 1.14). Other therapeutic areas, such as treatment with harvested and manipulated dendritic cells, mesenchymal cells, T cells and antigen-presenting cells, have progressed far more slowly. Nevertheless, with advances in gene engineering, the potential to treat illnesses with autologous re-engineered cellular therapies is very bright. Chapters 41–46 present a detailed account of the current state of the art in cellular therapies as well as a glimpse of where this field is heading.

Figure 1.14 Cryopreservation in liquid nitrogen.

The Future

This sixth edition of this textbook concludes, as have previous editions, with reflections on the future of the field. While speculation on the future is never easy, our own view is that the ability to perform targeted gene editing is one of the most promising current research endeavours. CRISPR (clustered regularly interspaced short palindromic repeats) technology allows for the targeted excision of DNA at any known sequence (Figure 1.15).

Figure 1.15 CRISPR technology allows targeted excision of DNA. *Source:* Tobias Arhelger / Adobe Stock.

Short tandem repeat DNA sequences (eventually renamed CRISPR) were originally discovered as part of the normal bacterial defence against viruses. Several genes in bacteria, called CRISPR-associated genes (cas), were found to code for nucleases specific for these repeat sequences, thereby disrupting viral genomes within bacteria.

One of these cas genes, *Cas9*, was found to work efficiently within eukaryotic cells as a nuclease that could be guided by RNA to a specific DNA target. This RNA guide can be synthesised to match the cellular DNA area of choice. By delivering the *Cas9* nuclease and the guiding RNA into a cell, the genome of that cell can be disrupted or edited in a controlled manner. The development of this remarkable technology resulted in the award of the 2020 Nobel Prize in Chemistry to Emmanuelle Charpentier and Jennifer Doudna.

One example of the application of CRISPR technology has focused on haemoglobin F production [9]. The *BCL11A* gene is the natural suppressor of haemoglobin F. *BCL11A* is turned on after birth, resulting in active downregulation of haemoglobin F transcription. CRISPR technology has been used to disrupt the promoter region of the *BCL11A* gene, thus removing its suppression, with a resulting increase in haemoglobin F production. In 2021, this approach was successfully applied to patients with sickle cell disease and beta-thalassemia, resulting in a dramatic decrease in painful crises in sickle cell patients and a decrease in transfusion dependence among those with thalassemia. The stunning application of the technology resulted in a phenotype cure previously unimaginable among patients with these lethal haematological disorders.

Conclusion

James Blundell would immediately recognise a red cell transfusion if he saw one today. However, the great part of what we do would be incomprehensibly advanced and far beyond his understanding. In a similar way, the technologies of the future will revolutionise medical care in ways we can hardly imagine. Let us look forward to a time when we can reflect back on non-specific immune suppression, apheresis therapy, blood group incompatibilities and one-dimensional laboratory triggers for transfusion care as practices that we needed to understand today so that we could achieve the promise of tomorrow.

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2 Essential Immunology for Transfusion Medicine

Gregory A. Denomme

Versiti Blood Research Institute and Diagnostic Laboratories, Milwaukee, WI, USA

Cellular Basis of the Immune Response

Leucocytes from the myeloid and lymphoid lineage form the innate and adaptive arms of the immune system. Each cell type has its own unique functions.

Innate Immune Cells

Phagocytes and Antigen-Presenting Cells

The innate immune system comprises the skin (epithelial) and mucous membrane barrier, lysozymes such as in the eye, phagocytic cells and inflammatory-related proteins (complement, C-reactive protein and lectins). The innate immune system has evolved to be the first line of defence to pathogens, to eliminate pathogens directly via lysozymes, by phagocytosis or direct complement lysis, and to stimulate the adaptive immune system to respond [1].

Monocyte-derived macrophages, neutrophils (polymorphonuclear neutrophils, PMNs) and dendritic cells (DCs) function as phagocytes that remove dead cells and cell debris or immune complexes. In addition, these cells act as the first line of innate defence, ingesting and clearing pathogens. The first step as an infectious agent breaks the skin barrier through a wound or otherwise is the recognition of pathogen-derived signals via the expression of pathogen-associated molecular patterns (PAMPs) or danger-associated molecular pattern (DAMP) signals from inflamed tissues via pattern recognition receptors (PRRs) [2]. In humans, pathogen recognition receptors detect distinct evolutionarily conserved moieties on pathogens. Recognition by these receptors triggers cell differentiation and expression or secretion of signalling proteins. Some of these proteins, termed cytokines, such as interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF), signal the body to increase acute-phase proteins that activate complement, while other chemokines attract circulating immune cells to the site of infection. The complement system can be activated by innate mechanisms via the expression of conserved pathogen carbohydrate motifs (PAMP recognition) or by the alternative pathway.

DCs and macrophages response to these cytokine signals and serve as antigen-presenting cells (APCs) that present digested linear protein peptide as antigen to specific T cells of the lymphoid lineage. PRR ligation of DCs and macrophages in this setting induces maturation of these cells into APCs with the acquisition of chemokine receptors that trigger their migration to lymph nodes where resting T cells reside. Simultaneously, mature APCs acquire co-stimulatory molecules and secrete other cytokines. These cellular changes are needed for T-cell activation and differentiation and eventually the immune

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response, including cytotoxic T cell and B cell antibody production in response to specific pathogens. The type of PRR ligation determines the cytokines produced, which in turn induces the optimal pathogen class-specific immune response.

Adaptive Immune Cells

T Lymphocytes

Specific cells of the immune system are involved in the adaptive immune response [3]. T cells are formed in the thymus. Through gene recombination, billions of different antigen receptor variations form. Each lymphocyte expresses a unique T-cell receptor (TCR). Immature T cells initially express a TCR in complex with CD4 or CD8 molecules, which respectively interact with major histocompatibility complex (MHC) class II or class I molecules on APCs. The presentation of self-antigens within such MHC molecules on thymic stromal cells determines the fate of the immature T cells. Interaction of T cells with APCs presenting self-antigens in the thymus results in the removal of T cells that have a TCR with high binding affinity for a self-antigen MHC. The T cells that survive this so-called negative selection process migrate to the secondary lymphoid organs, and are available to respond to foreign antigen peptides. There, TCR-specific binding to complexes of MHC can activate them with non-self (e.g. pathogen-derived) antigens on matured APCs. Interactions between the co-stimulatory molecules CD80 and CD86 on the APC with CD28 on the T cell subsequently drive the activated T cells into proliferation. Without co-stimulation (e.g. by not fully differentiated APCs or by insufficient or absent PRR ligation), T cells become non-functional (anergised). The requirement of PRR-induced danger signals thus forms a second checkpoint of T-cell activation to prevent reactivity to self-antigens. Hence, the normal removal of autologous apoptotic or dead cells and cell debris by phagocytes will not lead to alloimmunisation, since the PRR-induced signal is absent.

While immunoglobulins bind to amino acids in the context of the tertiary structure of the antigen, the TCR recognises linear amino acids of the antigen in the context of a foreign peptide bound to an MHC molecule. MHC characteristics ensure near-endless protein/peptide binding capacities and thus can continually respond to new and rapidly evolving pathogens. MHC class I is expressed on all nucleated cells and presents so-called endogenous antigen-constituting self-antigens, thus maintaining tolerance to self while also presenting antigens from viruses and other pathogens that use the replication machinery of eukaryotic cells for their propagation. The exceptions are viruses and parasites (like *Plasmodium falciparum*) that hide in red blood cells (RBCs), because the latter lack MHC. Fortunately, RBCs also lack the DNA replication machinery for such pathogens.

MHC class II molecules of APCs present antigenic proteins that are ingested or endocytosed from the extracellular milieu. The described antigen expression routes, however, are not absolute. Specialised DCs in this respect can also express pathogen-derived proteins that have been taken up by the DCs via the endocytic route and other extracellular-derived proteins on MHC class I to CD8+ cytotoxic T lymphocytes (CTLs). Conversely, cytosolic proteins can become localised in the endocytic system via the process of autophagy and become expressed in MHC class II.

Paradoxically, the fact that T cells become activated only when the specific TCR recognises non-self-antigens in the context of its own MHC (termed MHC restriction) seems to refute the condition whereby MHC/HLA-mismatched tissue transplants are rejected. However, upwards of 10% of T cells can be activated by donor-specific MHC; an additional alloantigen is not needed for this. This large circulating pool of T cells reacting with non-self MHC is usually present and explains the acute CD8-dependent rejection of non-self MHC in transplant rejection that occurs without previous immunisation.

T-Helper (Th) Cells

The activation of T cells as outlined in the previous section can result in their differentiation into Th cells, which is dependent on cytokines and plasma membrane molecules derived from the APC. Different Th subsets can be characterised by their cytokine release and their action in infected tissues. Th1 cells that release interferon (IFN)-gamma and IL-2 aid in killing intracellular pathogens upon cognate (i.e. antigen-specific) recognition of the macrophage. These differentiated T-helper cells are termed cytotoxic T lymphocytes. Th2 cells support B-cell differentiation and the formation of antibodies. These IL-4-, IL-5- and IL-13-releasing Th2 cells, furthermore, help to kill parasites by inducing immunoglobulin (Ig)E production, which activates mast cells, basophils and eosinophils. Th17 cells have been characterised and provide the host with protection against bacteria and fungi. These cells release IL-17 and IL-6 and are thought to enhance the early innate response by activating granulocytes, which are effective in antifungal immunity. Both Th1 and Th17 cells are drivers of strong pro-inflammatory immune responses, but also induce (partly) localised tissue damage, which might explain the association of Th1 with autoimmune diseases.

The recently defined follicular T-helper cells (Tfh) have now been recognised as the main CD4 T-cell subset that supports induction and regulation of humoral immunity (antibody responses). They are required to induce IgG and IgE antibody formation and to generate long-lived immunity via induction of long-lived plasma cells and memory B cells upon primary immunisation, and upon reactivation of memory B cells in the case of antigen re-encounter.

B Lymphocytes

In the bone marrow, progenitor B cells divide upon local cytokine signals from stromal cells, and are directed towards expression of their antigen-specific B-cell receptor (BCR). The specificity of a BCR is due to immunoglobulin heavy and light chain gene arrangements to create millions of specificities with different binding affinities of the surface-expressed immunoglobulin. Immature B-cell clones that show high binding affinity for self can be eliminated by premature stimulation, but many BCRs to self-antigens remain and normally are not expanded due to the lack of T help (thymic deletion). B cells mature in the peripheral lymphoid tissues, where they respond to foreign antigens via activation of the BCR. It is possible that initial B cells recruited by foreign antigens can bind to self-antigens and produce autoantibodies, as seen in immune thrombocytopenic purpura or warm autoimmune haemolytic anaemia [4]. Upon additional survival signals, these B cells proliferate and differentiate into short-lived or long-lived plasma cells, which in their turn secrete immunoglobulins with binding specificities like the activated B cells they are derived from. With T-cell help and presentation of antigen via APCs, these B cells can further mutate the complementarity-determining regions (CDRs) during proliferation and differentiation to gain higher antibody affinity for the foreign antigen. Depending on their differentiation pathway, it is plasma cells that lose BCR expression but secrete specific classes of antibodies (i.e. IgM, IgD, IgG, IgA and IgE). In addition to plasma cells, memory B cells are formed and persist long term, awaiting reactivation in a subsequent infection.

B-Cell Activation and T-Cell-Dependent Antibody Formation

B cells can also present antigen. The APC function of B cells is primarily designed to recruit specific Th cells that have previously become activated by DCs that have presented the same antigen. This process ensures that Th cells only support B-cell differentiation of those B cells that have become activated by the same pathogen, thus minimising the risk of activation of autoreactive B cells. Activated Th cells express CD40L, which provides co-stimulation to the B cells. Ligation of the

B cell via the CD40 co-stimulatory molecule, together with cytokines secreted by the Th cells, modulates the direction of B-cell differentiation. The main Th subset helping B-cell differentiation is the follicular T-helper cells (Tfh). In addition, these support the generation of class-switched B cells, which no longer express IgM but secrete immunoglobulins of the IgG or IgE class antibodies (see below). Some pathogens that have a repetitive structure (called thymus-independent antigens) can activate B cells to produce IgM antibodies against mostly extracellular pathogens without T-cell help. This offers a fast response mechanism, but is of low affinity. Higher-affinity antibody formation requires T-cell helper interactions.

Humoral Immune Response

Immunoglobulins are the secreted form of the BCR. These antigen-specific effector molecules are secreted by plasma cells. The basic monomeric structure of immunoglobulin is a roughly Y-shaped molecule made up of two identical heavy chains with four or five domains and two identical (kappa or lambda) light chains with two domains. Immunoglobulins are bivalent, two identical highly specific N-terminal antigen-binding sites, are the result of random heavy and light chain assortment and form the variable fragment antibody (Fab) domain of the molecule. The Fab domain forms the tertiary protein structure, with an almost unlimited repertoire of different three-dimensional 'specificities' for antigens (Figure 2.1). The mid (CH2) to C-terminal (CH3) region domains of two heavy chains combine and form the so-called fragment crystalline (Fc) region, which is more or less flexibly attached to the constant and variable regions by a so-called hinge area containing disulfide bonds between the two heavy chains. The Fc region determines the immunoglobulin class and consequently the imunoglobulin effector function, which is different for each immunoglobulin class. Some effector immunoglobulins form higher-order structures, with secreted IgA being a dimer and IgM a pentamer.

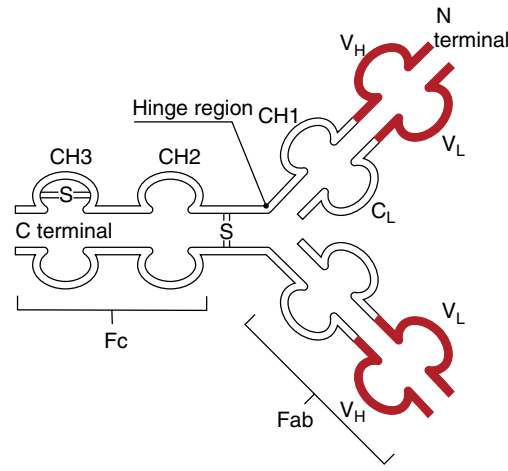

Figure 2.1 Basic structure of an immunoglobulin molecule. Domains are held in shape by disulfide bonds, though only one is shown. CHI–3, constant domains of an H chain; Cl, constant domain of a light chain; V_H, variable domain of an H chain; V_l, variable domain of a light chain.

Basis of Antibody Variability

Diversification of B cells takes place in extrafollicular tissues or in the germinal centres of the lymphoid organs with the help of CD4 T-helper cell-derived signals [5]. The BCR/antibody variability originates from the random recombination of heavy and light chain variable region genes: the heavy chain has three (VH, D and J) and the light chain has two (VL, J) genes, resulting in an enormous B-cell repertoire. Point mutations and insertions/deletions of nucleotides between the VH-D, D-J and VH-J regions, or so-called somatic hypermutations (SHMs) of the variable regions, result in the generation of some B cells with higher affinity for antigen. Thus, antibody production during the immune response results in antibody 'maturation' for non-self-antigens, while avoiding the expansion of antibodies with affinity for self-antigens due to a lack of self-reactive Th cells.

The process of affinity maturation leads to selection and survival of those B cells with a

BCR type that has the highest affinity for the antigen. Immunoglobulin class switching is induced by helper T-cell-released cytokines inducing the transcription of so-called switch regions. This process enables IgM by naive B cells to evolve into IgG or another class. IgG and IgA have subclasses that determine additional effector functions as well as their half-life and their ability for placental transfer (Table 2.1). The simultaneous regulation of SHM, affinity maturation and class switching explains why, during immune responses, the initial IgM generally show low binding affinity to the antigen, while those that are formed later on show enhanced antigen binding.

Antibody Effector Functions

While IgM only functions in circulation, IgA in this respect is mostly localised in epithelial tissues like the gut and exocrine (e.g. milk-, saliva- and tear-producing) glands. IgA acts as an early defence to pathogen invasion of these tissues and of the newborn via the mother's milk. Apart from the class, Ig functionality can also be modulated by glycosylation. Particularly for the C-terminal region of the IgG heavy chain but also for the Fab-binding region, glycosylation can accommodate different extensions of N-acetylglucosamine and mannose residues by galactose, sialic acid and so on. The extent and composition of these are influenced by many factors, including cytokines, age, pregnancy, hormones and bacterial DNA, and determine the stability and binding characteristics of the IgG. The knowledge in this area will be of major importance for engineering monoclonal antibodies and immunoglobulin preparations [6]. Finally, antibody specificities largely depend on the molecular structure of the VH and VL genes. In some instances, V genes are selectively used in antibody production against a certain antigen, as was found in pregnancy-induced rhesus D (RhD)-immunised females who volunteered for further immunisation with RhD [7].

Antibodies can neutralise toxins and pathogens. However, the clearing of these pathogens from the body is achieved by the following processes:

- For pathogens, an IgG-mediated process is responsible for the clearance of antigen-Ig complexes from circulation by resident phagocytes in the spleen and liver.
- For parasites, by exocytosis of stored mediators, e.g. lysozymes secreted from mast cells that are triggered by their Fc epsilon receptor recognising the Fc region of IgE.
- Activation of the complement cascade. This system is part of innate immunity, but is also vital to the effector functions of complement-fixing immunoglobulin isotypes. Central to the complement's function is the activation of C3 by three routes: the classical, alternative and lectin pathways.

The fate of antibody-coated cells, and more specifically RBCs in auto- or alloimmune-mediated haemolysis, is dependent on whether there is partial or total activation of the cascade downstream from C3. Total activation in this respect generates the membrane attack complex with the formation of the trimolecular complex of C4b2a3b or C5 convertase. This complex cleaves C5 into two fragments, C5a and C5b. C5b forms a complex with C6, C7 and C8, which facilitates the insertion of a number of C9 molecules in the membrane. This so-called membrane attack complex (MAC) creates pores in the membrane that destroy the target cell via lysis; the cell is unable to maintain isotonicity. IgM mediates this process especially well. MAC can also be transferred to cells close by and leads to so-called bystander lysis. Complement activation to C4b2a3b, in contrast, recruits phagocytes to sites of infection and mediates complement receptor clearance of the complement-coated cells. Complement-coated circulating cells are also cleared by macrophages in the spleen and liver.

Table 2.1 Immunoglobulin classes and their functions.

Structure			Function			
Isotype	**Heavy chain**	**Light chain**	**Configuration**	**Complement activation***	**Cells reacting with FcR**	**Placental passage**
IgM	μ	κ,λ	Pentamer	+++	L	–
IgGl	γl	κ,λ	Monomer	++	M, N, P, L, E	+++
IgG2	γ2	κ,λ	Monomer	+	P, L	+
IgG3	γ3	κ,λ	Monomer	+++	M, N, P, L, E	++
IgG4	γ4	κ,λ	Monomer	–	N, L, P	++
IgAl	αl	κ,λ	Monomer	+	–	–
IgA2	α2	κ,λ	Dimer in secretion	–	–	–
IgD	δ	κ,λ	Monomer	–	–	–
IgE	ε	κ,λ	Monomer	–	B, E, L	–

*Classical pathway.
B, basophils/mast cells; E, eosinophils; FcR, Fc receptors; Ig, immunoglobulin; L, lymphocytes; M, macrophages; N, neutrophils; P, platelets.

Red Blood Cell Antibodies Illustrating These Principles

Over 360 blood group antigens have been identified. Alloimmunisation can happen after contact with non-self RBC antigens by transfusion, transplantation, or during pregnancy and delivery, but can in some instances be elicited by contact with blood-borne antigens expressing structural moieties similar to some blood group antigens. While the cellular mechanisms are still largely unclear [6,8], the humoral response is easy to investigate through direct analyses of antibodies. IgM form first but are transient, in part since T-cell-independent B-cell-activated IgM-producing plasma cells can be short-lived. Alternatively, B-cell activation and secretion of IgM occur early in T-helper cell-dependent immune responses. So-called naturally occurring IgM can also be demonstrated, suggesting that they are derived from plasma cells or that they are formed upon continuous turnover of B cells into antibody-secreting plasma cells. The best-known IgM are those directed against the A or B blood group antigens, which are likely stimulated by exposure to gastrointestinal bacteria bearing A- and B-like non-self-antigenic structures. This explains their presence as early as the first few months of life.

Although some IgM to IgG switching does occur for the antibodies against the A and B antigens, the T-cell-independent antibody formation for these carbohydrate antigens has to be discerned from the thymic or T-cell-dependent high-affinity IgG-forming mechanisms against other blood group antigens.

Fortunately, A and B antigens are only expressed at low levels on fetal RBCs. Therefore, while the natural IgM cannot cross the placenta, anti-A or -B IgG transferred from the mother's blood usually do not lead to immune system effector mechanisms (phagocytosis or complement-mediated haemolysis) in the fetus.

Antibody and Complement-Mediated Blood Cell Destruction

A blood transfusion to a recipient with circulating antibodies against antigens expressed on the donor RBCs can cause acute (within 24 hours) or delayed haemolytic reactions. As the acute form can be life-threatening, especially when intravascular haemolysis is induced, the delayed form is typically less severe [8].

Most blood group allo- and autoantibodies of the IgG class bring about lysis via the interaction of the IgG constant domain with Fc gamma receptors on cells of the mononuclear phagocytic system.

- Fc gamma RI is the most important receptor that causes red cell destruction. This is a high-affinity receptor found predominantly on monocytes. The consequences of adherence of IgG-coated red cells to Fc gamma Rl-positive cells are phagocytosis and extravascular lysis.
- Fc gamma RII is a lower-affinity receptor found on monocytes, neutrophils, eosinophils, platelets and B cells.
- Fc gamma RIII is also of relatively low affinity and is found on macrophages, neutrophils, eosinophils and natural killer (NK) cells.
- There is also a neonatal Fc receptor (FcRn), expressed on the placenta and found in a soluble form in plasma, which mediates the transfer of IgG into the fetus and is involved in the control of IgG half-life.

The severity of haemolysis by IgG antibodies is determined by the concentration of antibody, its affinity for the antigen, the antigen density, the IgG subclass and the complement-activating capacity. IgG1 and IgG3 reduce RBC survival and, although IgG2 generally do not reduce survival, a specific Fc gamma receptor II isotype has increased affinity for IgG2.

The complement system, either working alone or in concert with an antibody, plays an important part in immune red cell destruction.

In contrast to extravascular Fc gamma receptor-mediated destruction, complement-mediated lysis occurs in the intravascular compartment. The ensuing release of anaphylatoxins such as C3a and C5a contributes to acute systemic effects. IgM anti-A and -B can cause such potentially lethal effects should an error in patient identification or ABO typing occur.

Red cells coated with C3b undergo extravascular haemolysis via complement receptor 1 (CR1/CD35) expressed by macrophages. If, however, the bound C3b degrades to its inactive components iC3b and C3dg, then the cell is effectively protected from lysis. Membrane-bound molecules such as decay-accelerating factor (DAF) and membrane inhibitor of reactive lysis (MIRL) expressed on the target cell, such as RBCs, also protect red cells from lysis by degrading C3b to its inactive forms.

Clinical Aspects Related to Alloimmunisation against Blood Cell Antigens

The incidence of RBC alloimmunisation has been reported to vary between 2% and 21% in recipients who are not chronically transfused. This reported variation is certainly influenced by the type and number of transfusions [9]. On the other hand, medication-suppressed immunity [10], an immune system activated by the presence of autoimmune disorders, infection [11] or preexisting haemolysis priming APCs with danger signals are all likely to influence immunisation efficacy. Finally, alloimmunisation efficacy is influenced by the genetic or ethnic differences between donor and patient. The latter is not only the case for blood group antigens, but also for HLA differences between donor and recipient. Certain HLA types are associated with a higher RBC alloimmunisation risk, suggesting specific HLA restriction for the presentation of some red cell antigens [12,13]. The fact that the first alloimmunisation increases the risk of further antibody formation might indicate the existence of a group of so-called responder patients, who have an intrinsically higher risk for alloimmunisation [14]. Better identification of clinical or genetic patient factors for red cell antigen alloimmunisation will be of great importance; this might enable cost-effective matching in specific high-risk conditions.

The immediate documentation of newly detected antibodies, and perhaps screening for antibodies after transfusion, is essential, because antibodies evanesce (become undetectable) over time. New antigen exposure, for example via a new transfusion, typically boosts their re-emergence and is the cause of delayed transfusion reactions that can lead to serious haemolysis. The rate of antibody evanescence is inversely proportionate to the strength, i.e. the antibody titre: the higher the initial antibody titre, the longer the antibody persists. The rate is also dependent on the nature of antigen exposure [15].

Although alloimmunisation against RBC antigens is important, (co-)transfused platelets and leucocytes, respectively expressing MHC class I and both class I and II, are more effective in inducing alloimmunisation [16]. HLA and antibodies to human platelet antigens (HPAs) are associated with various subsequent effects. First, HLA antibodies can cause refractoriness to platelet transfusions because donor platelets express varying amounts of HLA class I molecules. HPA antibodies can cause platelet refractoriness, but are more often the cause of neonatal alloimmune thrombocytopenia. HLA antibodies in this respect do not seem able to cross the placental barrier. However, they are partly instrumental in causing transfusion-related acute lung injury (TRALI). Finally, HLA antibodies in the recipient can cause cytokine-induced febrile non-haemolytic transfusion reactions when reacting with and destroying donor platelets or leucocytes. Mechanistically less clearly,

posttransfusion purpura and hyperhaemolysis [17] are also associated with antibodies against transfused blood components, but result in recipient cell destruction [18].

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KEY POINTS

1) Allogeneic blood is intrinsically non-self and capable of eliciting an immune response to foreign antigens; additional danger signals (as in inflammatory conditions) are needed to prime and activate the immune cells that are most important for alloantibody formation.
2) The ability of antibodies to bring about erythrocyte or platelet destruction varies according to their isotype and their antigenic, Fc receptor and complement-binding and -activating capacities. Glycosylation can affect antibody effector functionality.
3) Many clinical problems encountered in transfusion medicine are antibody based; in many cases, the causal mechanisms still need more elucidation [19].
4) Better identification of high-risk patients (responders) who are more likely to become alloimmunised, together with the increasing availability of donor red blood cell and platelet genotyping, will enable selective preventive and cost-effective donor–recipient matching [19].
5) High-risk alloimmunisation and conditions requiring chronic transfusion might benefit from immunomodulatory therapies shown to be preventing alloimmunisation.

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3 Human Blood Group Systems

Geoff Daniels[1] *and Sean Stowell*[2]

[1]*International Blood Group Reference Laboratory, NHS Blood and Transplant, Bristol, UK*
[2]*Joint Program in Transfusion Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*

A blood group may be defined as an inherited character of the red cell surface detected by a specific alloantibody. This definition would not receive universal acceptance, since cell surface antigens on platelets and leucocytes might also be considered blood groups, as might uninherited characters on red cells defined by autoantibodies or xenoantibodies. The definition is suitable, however, for the purposes of this chapter.

Most blood groups are organised into blood group systems. Each system represents a single gene or a cluster of two or more closely linked homologous genes. Of the 347 blood group specificities recognised by the International Society for Blood Transfusion, 303 belong to one of 36 systems (Table 3.1). All these systems represent a single gene, apart from Rh, Xg and Chido/Rodgers, which have two closely linked homologous genes, and MNS with three genes [1, 2].

Most blood group antigens are proteins or glycoproteins, with the blood group specificity determined primarily by the amino acid sequence, and most of the blood group polymorphisms result from single amino acid substitutions, though there are many exceptions. Some of these proteins cross the membrane once, with either the N-terminal or C-terminal outside the membrane, some cross the membrane several times and some are outside the membrane to which they are attached by a glycosylphosphatidylinositol anchor.

Some blood group antigens, including those of the ABO, P1PK, Lewis, H and I systems, are carbohydrate structures on glycoproteins and glycolipids. These antigens are not produced directly by the genes controlling their polymorphisms, but by genes encoding transferase enzymes that catalyse the final biosynthetic stage of an oligosaccharide chain.

The ABO System

ABO is often referred to as a histo-blood group system because, in addition to being expressed on red cells, ABO antigens are present on most tissues and in soluble form in secretions. At its most basic level, the ABO system consists of two antigens, A and B, indirectly encoded by two alleles, *A* and *B*, of the *ABO* gene. A third allele, O, produces neither A nor B. These three alleles combine to effect four phenotypes: A, B, AB and O (Table 3.2).

Clinical Significance

Two key factors make ABO the most important blood group system in transfusion medicine. First, the blood of almost all

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Table 3.1 Human blood group systems.

Number	Name	Symbol	Number of antigens	Gene symbol(s)	Chromosome
001	ABO	ABO	4	*ABO*	9
002	MNS	MNS	48	*GYPA, GYPB, GYPE*	4
003	P1PK	P1PK	3	*A4GALT*	22
004	Rh	RH	54	*RHD, RHCE*	l
005	Lutheran	LU	22	*BCAM*	19
006	Kell	KEL	35	*KEL*	7
007	Lewis	LE	6	*FUT3*	19
008	Duffy	FY	5	*DARC*	l
009	Kidd	JK	3	*SLC14A1*	18
010	Diego	DI	22	*SLC4A1*	17
011	Yt	YT	2	*ACHE*	7
012	Xg	XG	2	*XG,CD99*	X/Y
013	Scianna	SC	7	*ERMAP*	1
014	Dombrock	DO	10	*ART4*	12
015	Colton	CO	4	*AQP1*	7
016	Landsteiner–Wiener	LW	3	*ICAM4*	19
017	Chido/Rodgers	CH/RG	9	*C4A, C4B*	6
013	H	H	1	*FUT1*	19
019	Kx	XK	1	*XK*	X
020	Girbich	GE	11	*GYPC*	2
021	Cromer	CROM	18	*CD55*	l
022	Knops	KN	9	*CR1*	l
023	Indian	IN	4	*CD44*	11
024	Ok	OK	3	*BSG*	19

025	Raph	RAPH	1	*CD151*	11
026	John Milton Hagen	JMH	6	*SEMA7A*	15
027	I	I	1	*GCNT2*	6
028	Globoside	GLOB	2	*B3GALT3*	3
029	Gill	GIL	1	*AQP3*	9
030	RHAG	RHAG	4	*RHAG*	6
031	Forssman	FORS	1	*GBGT1*	9
032	JR	JR	1	*ABCG2*	4
033	Lan	LAN	1	*ABCB6*	2
034	Vel	VEL	1	*SMIM1*	l
035	CD59	CD59	1	*ABCG2*	11
036	Augustine	AUG	4	*SLC29A1*	6
037	Kanno	KANNO	1	*PRNP*	20
038	SID	SID	1	*B4GALNT2*	17
039	CTL2	CTL2	2	*SLC44A2*	19
040	PEL	PEL	1	*ABCC4*	13
041	MAM	MAM	1	*EMP3*	19
042	EMM	EMM	1	*PIGG*	4
043	ABCC1	ABCC1	1	*ABCC1*	16

Table 3.2 The ABO system.

		Frequency			
Phenotype	**Genotypes**	**Europeans***	**Africans†**	**Indians§**	**Antibodies present**
O	*O/O*	43%	51%	31%	Anti-A, -B, -A,B
A_1	A^1/A^1,A^1/O,A^1/A^2	35%	18%	26%	Anti-B
A_2	A^2/A^2,A^2/O	10%	5%	3%	Sometimes anti-A_1
B	*B/B.B/O*	9%	21%	30%	Anti-A
A_1B	A^1/B	3%	2%	9%	None
A_2B	A^2/B	1%	1%	1%	Sometimes anti-A_1

*English donors.
†Donors from Kinshasa, Congo.
§Makar from Mumbai.

adults contains antibodies to those ABO antigens lacking from their red cells (see Table 3.2). In addition to anti-A and anti-B, group O individuals have anti-A,B, an antibody to a determinant common to A and B. Second, ABO antibodies are immunoglobulin (Ig)M, though they may also have an IgG component, have thermal activity at 37 °C, activate complement and cause immediate intravascular red cell destruction, which can give rise to severe and often fatal haemolytic transfusion reactions (HTRs) (see Chapter 8). Major ABO incompatibility (i.e. donor red cells with an ABO antigen not possessed by the recipient) must be avoided in transfusion and, ideally, ABO-matched blood (i.e. of the same ABO group) would be provided.

ABO antibodies seldom cause haemolytic disease of the fetus and newborn (HDFN) and when they do, it is usually mild.

Biosynthesis and Molecular Genetics

Red cell A and B antigens are expressed predominantly on oligosaccharide structures on integral membrane glycoproteins, but are also on glycosphingolipids embedded in the membrane. The tetrasaccharides that represent the predominant form of A and B antigens on red cells are shown in Figure 3.1, together with their biosynthetic precursor, the H antigen, which is abundant on group O red cells. The product of the *A* allele is a glycosyltransferase that catalyses the transfer of *N*-acetylgalactosamine (GalNAc) from a nucleotide donor substrate, UDP-GalNAc, to the fucosylated galactose (Gal) residue of the H antigen, the acceptor substrate. The product of the *B* allele catalyses the transfer of Gal from UDP-Gal to the fucosylated Gal residue of the H antigen. GalNAc and Gal are the immunodominant sugars of A and B antigens, respectively. The *O* allele produces no transferase, so the H antigen remains unmodified.

The *ABO* gene on chromosome 9 consists of seven exons. The *A* and *B* alleles differ by four nucleotides in exon 7 encoding amino acid substitutions. These determine whether the gene product is a GalNAc-transferase (A)

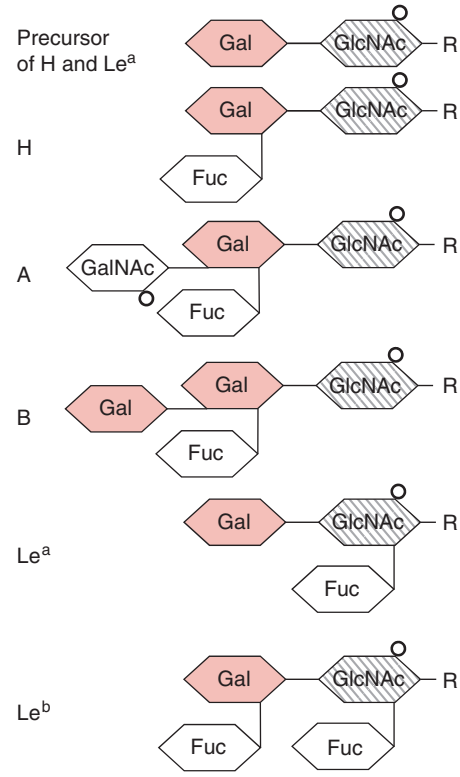

Figure 3.1 Diagram of the oligosaccharides representing H, A, B, Le^a and Le^b antigens and the biosynthetic precursor of Hand Le^a. R, remainder of molecule.

or Gal-transferase (B) [3]. The most common *O* allele (O^1) has an identical sequence to A, apart from a single nucleotide deletion in exon 6, which shifts the reading frame and introduces a translation stop codon before the region of the catalytic site, so that any protein produced would be truncated and have no enzyme activity [4,5].

H, the Biochemical Precursor of A and B

H antigen is the biochemical precursor of A and B (see Figure 3.1). It is synthesised by an α1,2-fucosyltransferase, which catalyses the transfer of fucose from its donor substrate to the terminal Gal residue of its acceptor substrate. Without this fucosylation, neither A nor B antigens can be made. Two genes, active in different tissues, produce α1,2-fucosyltransferases: *FUT1*, responsible for

H on red cells; *FUT2*, for H in many other tissues and in secretions. Homozygosity for inactivating mutations in *FUT1* leads to an absence of H from red cells and, therefore, an absence of red cell A or B, regardless of *ABO* genotype. Such mutations are rare, as are red cell H-deficient phenotypes. In contrast, inactivating mutations in *FUT2* are relatively common and about 20% of white Europeans (non-secretors) lack H, A and B from body secretions, despite expressing those antigens on their red cells. Very rare individuals who have H-deficient red cells and are also H non-secretors (Bombay phenotype) produce anti-H together with anti-A and -B, and this can cause a severe transfusion problem [4,5].

The Rh System

Rh is the most complex of the blood group systems, with many specificities. The most important of these is D (RH1).

Rh Genes and Proteins

The antigens of the Rh system are encoded by two genes, *RHD* and *RHCE*, which produce D and CcEe antigens, respectively. The genes are highly homologous, each consisting of 10 exons. They are closely linked, but in opposite orientations, on chromosome 1 (Figure 3.2) [6]. Each gene encodes a 417 amino acid polypeptide that differs by only 31–35 amino acids, according to Rh genotype. The Rh proteins are not glycosylated and span the red cell membrane 12 times, with both termini inside the cytosol and with 6 external loops, the potential sites of antigenic activity (see Figure 3.2).

D Antigen

The most significant Rh antigen clinically is D. About 85% of white people are D+ (Rh-positive) and 15% are D– (Rh-negative). In Africans, only about 3–5% are D–, and in East Asia D– is rare.

The D– phenotype is usually associated with the absence of the whole D protein from the red cell membrane. This explains why D is so immunogenic, as the D antigen comprises numerous epitopes on the external domains of the D protein. In white people, the D– phenotype almost always results from homozygosity for a complete deletion of *RHD*. D-positives are either homozygous or heterozygous for the presence of *RHD*. In Africans, in addition to the deletion of *RHD*, D– often results from an inactive *RHD* (called *RHD*ψ) containing translation stop codons within the reading frame.

Numerous variants of D are known, though most are rare [7,8]. They are often split into two types, partial D and weak D, though this dichotomy is not adequately defined and is of little value for making clinical decisions. Partial D antigens lack some or most of the D epitopes. If an individual with a partial D phenotype is immunised by red cells with a complete D antigen, they might make antibodies to those epitopes they lack. The D epitopes comprising

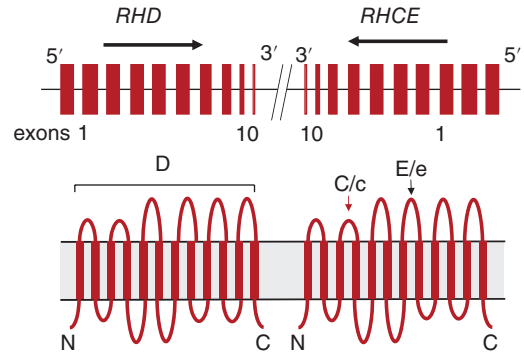

Figure 3.2 Diagrammatic representation of the Rh genes, *RHD* and *RHCE*, shown in opposite orientations as they appear on the chromosome, and of the two Rh proteins in their probable membrane conformation, with 12 membrane-spanning domains and 6 extracellular loops expressing D, C/c and E/e antigens.

partial D may be expressed weakly or may be of normal or even enhanced strength. Weak D antigens appear to express all epitopes of D, but at a lower site density than normal D. D variants result from amino acid substitutions in the D protein, occurring either as a result of one or more missense mutations in *RHD* or from one or more exons of *RHD* being exchanged for the equivalent exons of *RHCE* in a process called gene conversion.

Anti-D

Anti-D is almost never produced in D– individuals without exposure to D+ red cells. D is highly immunogenic and approximately 20% of D– recipients of transfused D+ red cells make anti-D. Anti-D can cause severe immediate or delayed HTRs and D+ blood must never be transfused to a patient with anti-D.

Anti-D is one of the most common causes of severe HDFN.

Prediction of Fetal Rh Phenotype by Molecular Methods

Knowledge of the molecular bases for D– phenotypes has made it possible to devise tests for predicting fetal D type from fetal DNA. This is a valuable tool in assessing whether the fetus of a woman with anti-D is at risk from HDFN [9]. Most methods involve polymerase chain reaction (PCR) tests that detect the presence or absence of *RHD*. The usual source of fetal DNA is the small quantity of free fetal DNA present in maternal plasma. This non-invasive form of fetal D typing is now provided as a service in many countries for alloimmunised D– women. In addition, in a few European countries non-invasive fetal *RHD* genotyping is offered to all D– pregnant women, so that only those with a D+ fetus receive routine antenatal anti-D prophylaxis (see Chapter 33).

C and c, E and e

C/c and E/e are two pairs of antigens representing alleles of *RHCE*. The fundamental difference between C and c is a serine–proline substitution at position 103 in the second external loop of the CcEe protein (see Figure 3.2), and E and e represent a proline–alanine substitution at position 226 in the fourth external loop [10].

Anti-c is clinically the most important Rh antibody after anti-D and may cause severe HDFN. On the other hand, anti-C, -E and -e rarely cause HDFN and when they do, the disease is generally mild, though all have the potential to cause severe disease.

Other Rh Antigens

Of the 54 Rh antigens, 20 are polymorphic, i.e. have a frequency between 1% and 99% in at least one major ethnic group, 22 are rare antigens and 12 are very common antigens. Antibodies to many of these antigens have proved to be clinically important and it is prudent to treat all Rh antibodies as being potentially clinically significant [11].

Other Blood Group Systems

Of the remaining blood group systems (see Table 3.1), the most important clinically are Kell, Duffy, Kidd and MNS.

Kell System

The original Kell antigen, K (KEL1), has a frequency of about 9% in Caucasians, but is rare in other ethnic groups. Its antithetical (allelic) antigen, k (KEL2), is common in all populations. The remainder of the Kell system consists of one triplet and five pairs of allelic antigens – Kp^a, Kp^b and Kp^c; Js^a and Js^b; K11 and K17; K14 and K24; VLAN and VONG; KYO and KYOR – plus 17 high-frequency and three low-frequency antigens. Almost all represent single amino acid substitutions in the Kell glycoprotein.

Anti-K can cause severe HTRs and HDFN. About 10% of K– patients who are given one unit of K+ blood produce anti-K, making K the next most immunogenic antigen after D. In most cases of HDFN caused by anti-K, the mother will have had previous blood transfusions. HDFN caused by anti-K differs from Rh

HDFN in that anti-K appears to cause fetal anaemia by suppression of erythropoiesis, rather than immune destruction of mature fetal erythrocytes [11]. Most other Kell system antibodies are rare and are best detected by an antiglobulin test.

The Kell antigens are located on a large glycoprotein, which belongs to a family of endopeptidases that process biologically important peptides, and is able to cleave the biologically inactive peptide big endothelin-3 to produce endothelin-3, an active vasoconstrictor.

Duffy System

Fy^a and Fy^b represent a single amino acid substitution in the extracellular N-terminal domain of the Duffy glycoprotein. Their incidence in Caucasians is Fy^a 68%, Fy^b 80%. But about 70% of African Americans and close to 100% of West Africans are Fy(a–b–) (Table 3.3).

Africans are homozygous for an *FY*B* allele containing a mutation in a binding site for the erythroid-specific GATA-1 transcription factor, which means that Duffy glycoprotein is not expressed in red cells, though it is present in other tissues (Table 3.4) [10,12]. The Duffy glycoprotein is the receptor exploited by *Plasmodium vivax* merozoites for penetration of erythroid cells. Consequently, the Fy(a–b–) phenotype confers resistance to *P. vivax* malaria. The Duffy glycoprotein (also called Duffy antigen chemokine receptor, DARC) is a red cell receptor for a variety of chemokines, including interleukin-8.

Anti-Fy^a is not infrequent and is found in previously transfused patients who have usually made other antibodies. Anti-Fy^b is very rare.

Table 3.3 The Duffy system: phenotypes and genotypes.

		Frequency (%)	
Phenotype	**Genotype**	**Europeans**	**Africans**
Fy(a+b–)	*FY*A/A* or *FY*A/Null*[†]	20	10
Fy(a+b+)	*FY*A/B*	48	3
Fy(a–b+)	*FY*B/B* or *FY*B/Null*	32	20
Fy(a–b–)	*FY*Null/Null*	0	67

[†]*Null* represents the allele that produces no Duffy antigens on red cells.

Table 3.4 Nucleotide polymorphisms in the promoter region and in exon 2 of the three common alleles of the Duffy gene.

Allele	**GATA box sequence 64–69 (promoter)**	**Codon 42 (exon 2)**	**Antigen**
*FY*A*	TTATCT	GGT (Gly)	Fy^a
*FY*B*	TTATCT	GAT (Asp)	Fy^b
*FY*Null*	TTACCT	GAT (Asp)	Red cells – none Other tissues – probably Fy^b

Both may cause acute or delayed HTRs and HDFN varying from mild to severe [11].

Kidd System

Kidd has two common alleles, *JK*A* and *JK*B*, which represent a single amino acid change in the Kidd glycoprotein. Both Jk^a and Jk^b antigens have frequencies of about 75% in Caucasian populations. A Kidd-null phenotype, Jk(a–b–), results from homozygosity for inactivating mutations in the Kidd gene, *SLC14A1*. It is very rare in most populations, but reaches an incidence of greater than 1% in Polynesians. The Kidd glycoprotein is a urea transporter in red cells and in renal endothelial cells.

Anti-Jk^a is uncommon and anti-Jk^b is very rare, but both cause severe transfusion reactions and, to a lesser extent, HDFN [11]. Kidd antibodies have often been implicated in delayed HTRs. They are typically difficult to detect serologically and tend to disappear rapidly after stimulation.

MNS System

MNS, with a total of 48 antigens, is second only to Rh in complexity. MNS antigens are present on one or both of two red cell membrane glycoproteins, glycophorin A (GPA) and glycophorin B (GPB). They are encoded by homologous genes, *GYPA* and *GYPB*, on chromosome 4.

The M and N antigens, both with frequencies of about 75%, differ by amino acids at positions 1 and 5 of the external N-terminus of GPA. S and s have frequencies of about 55% and 90%, respectively, in a Caucasian population and represent an amino acid substitution in GPB. About 2% of black West Africans and 1.5% of African Americans are S–s–, a phenotype virtually unknown in other ethnic groups, and most of these lack the U antigen, which is present when either S or s is expressed. The numerous MNS variants mostly result from amino acid substitutions in GPA or GPB and from hybrid GPA–GPB molecules, formed by intergenic recombination between *GYPA* and *GYPB*. The phenotypes resulting from these hybrid proteins are rare in Europeans and Africans, but the GP.Mur (previously Mi.III) variant phenotype occurs in up to 10% of East Asians. GPA and GPB are exploited as receptors by the malaria parasite *Plasmodium falciparum*.

Anti-M and -N are not generally clinically significant, though anti-M is occasionally haemolytic [9]. Anti-S, the rarer anti-s, and anti-U can cause HDFN and have been implicated in HTRs. Although rare elsewhere, anti-Mur, which detects red cells of the GP.Mur phenotype, is common in East Asian and Oceanic regions and causes severe HTRs and HDFN.

Biological and Clinical Significance of Blood Group Antigens

The functions of several red cell membrane protein structures bearing blood group antigenic determinants are known, or can be deduced from their structure. Some are membrane transporters, facilitating the transport of biologically important molecules through the lipid bilayer: band 3 membrane glycoprotein, the Diego antigen, provides an anion exchange channel for HCO_3^- and Cl^- ions; the Kidd glycoprotein is a urea transporter; the Colton glycoprotein is aquaporin 1, a water channel; the GIL antigen is aquaporin 3, a glycerol transporter; JR and Lan glycoproteins are probably porphyrin transporters; Augustine glycoprotein is a nucleoside transporter; and RhAG may form a carbon dioxide and, possibly, oxygen channel, and could function as an ammonia/ammonium transporter [13–15]. The Lutheran, LW and Indian (CD44) glycoproteins are adhesion molecules, possibly serving their primary functions during erythropoiesis. The MER2 antigen is located on the tetraspanin CD151, which associates with integrins within basement membranes, but its function on red cells is not known. The Duffy glycoprotein is a chemokine receptor and could function as a 'sink' or

scavenger for unwanted chemokines. The Cromer (CD55), Knops (CD35) and CD59 antigens are markers for complement regulatory proteins that protect the cells from destruction by autologous complement.

Some blood group glycoproteins appear to be enzymes, though their functions on red cells are not known: the Yt antigen is acetylcholinesterase, the Kell antigen is an endopeptidase and the sequence of the Dombrock glycoprotein suggests that it belongs to a family of adenosine diphosphate (ADP)-ribosyltransferases. The C-terminal domains of the Gerbich antigens, GPC and GPD, and the N-terminal domain of the Diego glycoprotein, band 3, are attached to components of the cytoskeleton and function to anchor it to the external membrane. The carbohydrate moieties of the membrane glycoproteins and glycolipids, especially those of the most abundant glycoproteins, band 3 and GPA, constitute the glycocalyx, an extracellular coat that protects the cell from mechanical damage and microbial attack.

The Rh proteins are associated as heterotrimers with the glycoprotein RhAG in the red cell membrane, and these trimers are part of a macrocomplex of red cell surface proteins that include tetramers of band 3 plus LW, GPA, GPB and CD47, and are linked to the red cell cytoskeleton through protein 4.2 and ankyrin. There is probably another protein complex containing Rh proteins and dimers of band 3, plus Kell, Kx and Duffy blood group proteins, and this is linked to the cytoskeleton through glycophorin C (Gerbich blood group), MMP1 and protein 4.1R.

The structural differences between antithetical red cell antigens (e.g. A and B, K and k, Fy^a and Fy^b) are small, often being just one monosaccharide or one amino acid. The biological importance of these differences is unknown and there is little evidence to suggest that the product of one allele confers any significant advantage over the other. Some blood group antigens are exploited by pathological microorganisms as receptors for attaching and entering cells, so in some cases absence or changes in these antigens could be beneficial. It is likely that interaction between cell surface molecules and pathological microorganisms has been a major factor in the evolution of blood group polymorphism.

The entire goal of detecting alloantibodies clinically is to avoid red blood cell (RBC) incompatibility that could lead to a haemolytic reaction following transfusion or to HDFN. However, surprisingly little is known regarding the factors that dictate whether alloantibody engagement of a particular alloantigen will actually result in haemolysis [16,17]. While alloantibody engagement of certain alloantigens is thought to be more likely to induce haemolytic reactions, intravascular or extravascular haemolysis is not the inevitable outcome of RBC alloantigen incompatibility. A variety of outcomes can occur following alloantibody engagement, including actual loss of the target alloantigen from the RBC surface in the absence of appreciable haemolysis, extravascular haemolysis, intravascular haemolysis or no appreciable change in RBC clearance or alloantigen loss. The parameters that govern the outcome of alloantibody engagement remain incompletely understood. However, variables such as the density of the alloantigen on the cell surface, the orientation of the target epitope, the lateral mobility of the alloantigen and the type of alloantibody that recognises these alloantigens likely influence this outcome [16,17]. While studies over a century have characterised and catalogued RBC alloantigens, future studies aimed at understanding the factors that dictate the consequences of alloantibody engagement of RBC alloantigens hold significant promise in providing additional strategies to prevent alloantibody-mediated haemolysis. Such strategies are especially important when patients present with life-threatening anaemia and no compatible RBCs are readily available. Understanding the pathophysiology of incompatible transfusion may also provide a

more targeted approach when treating haemolytic reactions [18,19]. Moreover, it is important to note that alloantibody-independent HTRs can also cause significant morbidity and mortality in patients with sickle cell disease. As a result, strategies aimed at proactively evaluating chronically transfused patients post transfusion for possible delayed HTRs in the presence or absence of alloantibodies may be necessary to accurately detect and then treat these reactions in at-risk patients [19].

KEY POINTS

1) The International Society of Blood Transfusion recognises 347 blood group specificities, 303 of which belong to one of 36 blood group systems.
2) The most important blood group systems clinically are ABO, Rh, Kell, Duffy, Kidd and MNS.
3) ABO antibodies are almost always present in adults lacking the corresponding antigens and can cause fatal intravascular haemolytic transfusion reactions.
4) ABO antigens are carbohydrate structures on glycoproteins and glycosphingolipids.
5) Anti-RhD is the most common cause of haemolytic disease of the fetus and newborn.
6) Red cell surface proteins serve a variety of functions, though many of their functions are still not known.
7) Predicting whether an alloantibody will cause red blood cell haemolysis remains difficult.

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4 Human Leukocyte Antigens

H. Cliff Sullivan[1] *and Caroline R. Alquist*[2]

[1] *HLA Laboratory, Director of Cellular Therapy, Center for Transfusion and Cellular Therapies, Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA, USA*
[2] *Transplantation Immunology & Therapeutic Apheresis, Hoxworth Blood Center, University of Cincinnati, Cincinnati, OH, USA*

The genes encoding for the human leucocyte antigens (HLAs) are located on the short (p, petite) arm of chromosome 6. Spanning 4 Mb, this genomic region is divided into three subregions (Figure 4.1):

- **Class I subregion** encodes heavy (α) chain of the classical (HLA-A, -B and -C) and non-classical (HLA-E, -F and -G) class I molecules.
- **Class II subregion** encodes classical HLA-DR, -DQ and -DP genes (both A [alpha] and B [beta] chains) and the non-classical HLA-DMA, -DMB, -DOA and -DOB genes.
- **Class III subregion** encodes a diverse group of proteins, including complement components (C4Bf), tumour necrosis factor (TNF) and heat-shock proteins (HSPs).

HLA Class I Genes

HLA class I genes are defined, according to their structure, expression and function, as classical (HLA-A, -B and -C) and non-classical (HLA-E, -F and -G). Both classical and non-classical HLA class I genes encode for a heavy (α) chain of approximately 43 kd. The expressed heavy chain is non-covalently linked to a non-polymorphic light chain, β_2-microglobulin, a protein encoded by a 12 kd gene on chromosome 15 (Figure 4.2). The extracellular portion of the heavy chain has three domains (α1, α2 and α3), approximately 90 amino acids long. These domains are encoded by exons 2, 3 and 4 of the class I gene, respectively. The α1 and α2 domains, encoded by exons 2 and 3, are the most polymorphic, forming a peptide-binding groove that can accommodate up to eight or nine amino acid long antigenic peptides.

HLA Class II Genes

The classical HLA class II genes (DRA, DRB, DQA, DQB, DPA and DPB) encode for A and B chains, approximately 34 and 28 kd, respectively. The expressed A and B protein chains non-covalently associate into a heterodimer. Each chain consists of two extracellular domains (α1/β1 and α2/β2), one transmembrane domain and one cytoplasmic domain (Figure 4.3). The α1/β1 and α2/β2 domains are encoded by exon 2 and exon 3 of the class II

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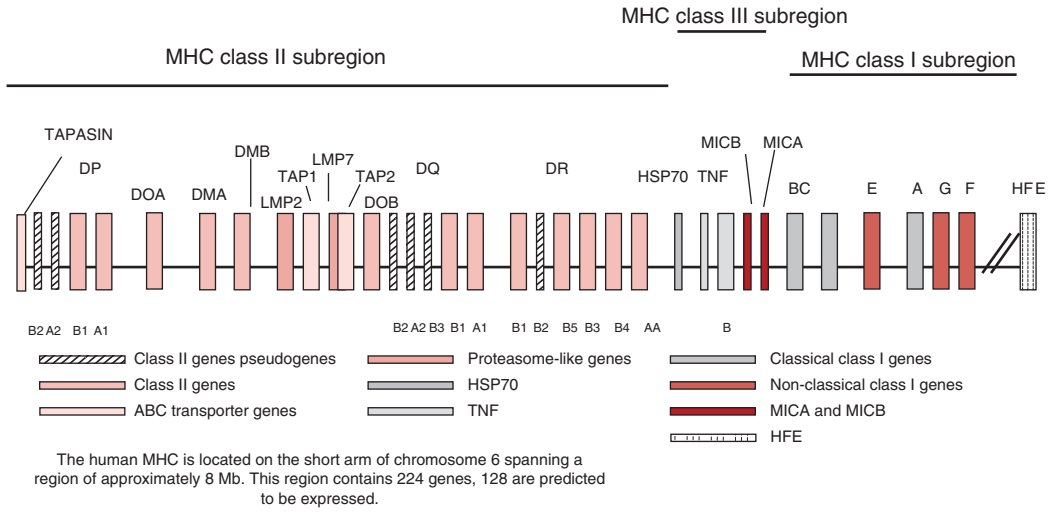

Figure 4.1 Map of the human leucocyte antigen complex. HSP, heat-shock protein; TNF, tumour necrosis factor. *Source:* Based on Trowsdale and Campbell 1997 [1].

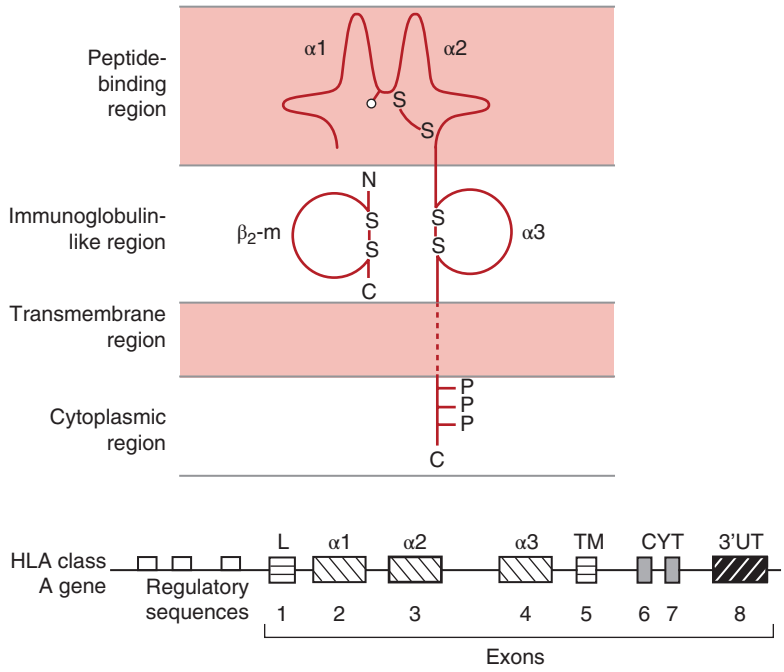

Figure 4.2 HLA class I molecule structure.

gene, respectively. The majority of genetic polymorphism is in the β1 domain of the DR molecules and in the α1 and β1 domains of the DQ and DP molecules. Like class I molecules, these domains also form a peptide-binding groove; however, the class II groove is open at both sides. This configuration can accommodate antigenic peptides of varying sizes, although most of them are 13–25 amino acids long.

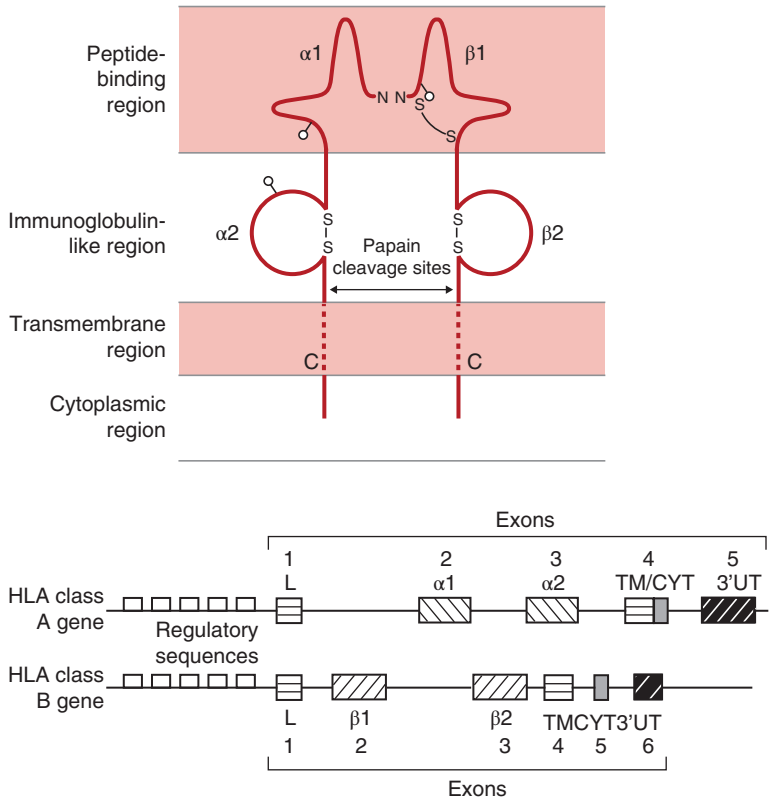

Figure 4.3 HLA class II molecule structure.

Genetic Organisation and Expression of HLA Class II Genes

There is one DRA gene of limited polymorphism and nine DRB genes, of which B1, B3, B4 and B5 are highly polymorphic and B2, B6 and B9 are pseudogenes. The main serologically defined DR specificities (DR1–DR18) are determined by the polymorphic DRB1 gene (Figure 4.4). The number of DRB genes expressed in everyone varies according to the *DRB1** allele expressed. In contrast to the DRB genes, there are two DQA and three DQB genes, but only DQA1 and DQB1 are expressed and both are polymorphic. Similarly, there are two DPA and two DPB genes, but only DPA1 and DPB1 are expressed and both are polymorphic.

Expression of HLA Molecules

The HLA class I molecules (A, B, C) are present on most tissues and blood cells, including T and B lymphocytes, granulocytes and platelets. Low levels of expression have been detected in endocrine tissue, skeletal muscle and cells of the central nervous system. The HLA class II molecules are constitutively expressed on B lymphocytes, monocytes and dendritic cells, but can also be detected on activated T lymphocytes and activated granulocytes. HLA class II expression can be induced in cells such as fibroblasts and endothelial cells via activation and/or the effect of certain inflammatory cytokines, such as interferon (IFN)-γ, TNF and interleukin (IL)-10. Both classical and

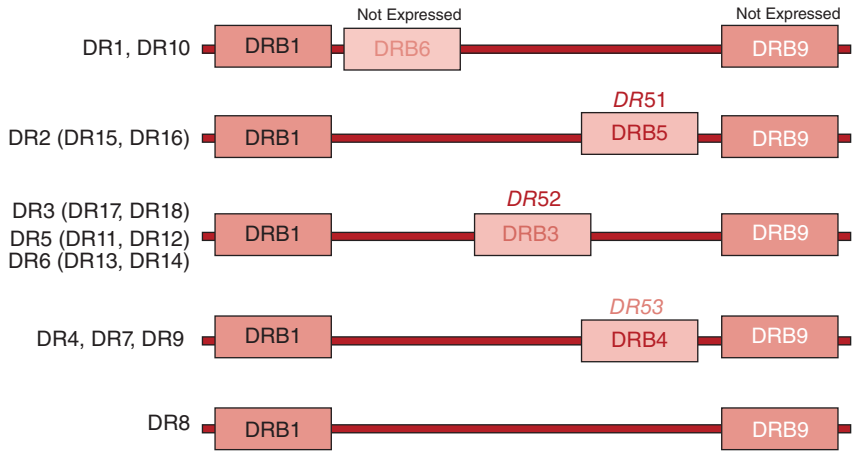

Figure 4.4 Expression of HLA-DRB genes. Notes: Some DR15 and DR16 haplotypes do not express DRB5. Rare associations include DR8 with DRB3 and DR1 with DRB5. Most DR7, DQ9 (DQB1*03:03) haplotypes do not express DRB4 (i.e. DR53 Null). Some DR4 haplotypes with DQ9 may also have the DR53 Null. *Source*: Courtesy of Robert A. Bray, PhD, D(ABHI).

non-classical HLA molecules can also be found in soluble forms and may play a role in the induction of peripheral tolerance.

Genetics

HLA genes, which are co-dominantly expressed and inherited in a Mendelian fashion, are highly polymorphic. Some alleles of these genes segregate in strong linkage disequilibrium (LD), meaning the observed frequency of alleles of different loci segregating together is greater than the frequency expected by random association. Some alleles and patterns of LD are noted across all populations, but others are associated with select populations.

The genetic region containing all HLA genes on a single chromosome 6 is termed the 'haplotype'. Some HLA haplotypes are also commonly found in diverse populations, whereas others are unique to a particular ethnic group.

Function of HLA Molecules

The main function of HLA molecules is to present antigenic peptides to T cells. Several co-stimulatory molecules (e.g. CD80 and CD86) and adhesion molecules such as ICAM-1 (CD54) and LFA-3 (CD58) also contribute to the interactions between HLA molecules, antigenic peptides and T-cell receptors.

HLA class I molecules are primarily, but not exclusively, involved in the presentation of endogenous antigenic peptides to CD8 cytotoxic T cells. Both the classical and non-classical HLA class I molecules also interact with a family of receptors present on natural killer (NK) cells: killer-inhibitory or killer-activating receptors belonging to either the killer immunoglobulin receptors (KIRs) superfamily or the C-type lectin superfamily, CD94-NKG2 [2].

The HLA class II molecules (DR, DQ and DP) are mostly involved in the presentation of exogenous antigenic peptides to CD4 helper T cells. Once activated, these CD4 cells can initiate and regulate a variety of processes leading to the maturation and differentiation of cellular (CD8 cytotoxic T cells) and humoral effectors (such as antibody production by plasma cells). Activated effectors also secrete pro-inflammatory cytokines (IL-2, IFN-γ, TNF-α) and regulatory cytokines (IL-4, IL-10 and transforming growth factor [TGF]-β).

Table 4.1 An example of a current HLA nomenclature.

HLA	HLA region
HLA-DR	Identifies the HLA locus
HLA-DR13	A serologically defined antigen
HLA-DRB1*	Identifies the HLA locus and gene. The asterisk indicates that the HLA allele(s) have been defined using DNA-based techniques
HLA-DRB1*13	A group of HLA alleles with a common DRB1*13 sequence, termed first field resolution (field before the first colon). First field resolution typing is often considered the serological equivalent of antigen family
HLA-DRB1*13:01	A specific HLA allele, termed second field resolution (numbers appear in the second field, after the first colon)
HLA-DRB1*13:01:02	An allele that differs by a synonymous (silent or non-coding) mutation. This is termed third field resolution
HLA-DRB1*13:01:01:02	An allele that contains a mutation outside the coding region. This is termed fourth field resolution

Source: Data from Nunes et al. 2011 [3].

HLA Nomenclature, Gene Polymorphism and Molecular HLA Typing

The HLA polymorphisms were initially identified and defined using serological and cellular techniques. The development of gene cloning and DNA sequencing has allowed a detailed analysis of these genes at the single nucleotide level, revealing the existence of locus-specific sequences in both coding (exons) and non-coding (introns) regions and the existence of common nucleotide sequences between several alleles of the same and/or different loci.

Due to the complexity of HLA polymorphism and the number of new alleles defined each year, a revised nomenclature has been implemented (Table 4.1) [4]. A tally of DNA-identified HLA alleles, expressed proteins and known nulls is shown in Table 4.2, but updated information is available at http://hla.alleles.org/nomenclature/stats.html [5].

The primary source of HLA genetic variation is located within the α1 and α2 domains for class I molecules and the α1 and β1

Table 4.2 Number of recognised HLA antigens/alleles.

Gene	Alleles	Protein	Nulls
HLA class I			
HLA-A	6192	3855	316
HLA-B	7431	4739	252
HLA-C	6067	3612	263
HLA-E	255	109	7
HLA-F	44	6	0
HLA-G	80	21	4
HLA class II			
HLA-DRB1	2737	1934	91
HLA-DRA1	29	2	0
HLA-DRB3	345	258	16
HLA-DRB4	166	110	19
HLA-DRB5	130	101	17
HLA-DQB1	1857	1232	79
HLA-DQA1	260	106	6
HLA-DPB1	1584	1037	82
HLA-DPA1	202	71	4

domains for class II molecules, termed hypervariable regions. Techniques developed to further characterise these polymorphisms using polymerase chain reactions (PCR) to amplify specific genes or regions for analysis include PCR sequence-specific priming (PCR-SSP), PCR sequence-specific oligonucleotide probing (PCR-SSOP), DNA sequencing-based typing using the Sanger method (SBT) and next-generation sequencing (NGS). The advantages and disadvantages of the various techniques are summarised in Table 4.3.

PCR Sequence-Specific Priming

This technique involves the use of specific primers designed to anneal with DNA solely at areas of interest, generally targeting the hypervariable regions to initiate synthesis. Amplification only occurs if the primer binds. Products of amplification can be visualised by agarose gel electrophoresis or by real-time PCR methods to detect the amplicons as they generate. Elimination of the electrophoresis step improves turnaround times, making real-time PCR methods well suited for time-sensitive deceased donor HLA typing [6]. However, as the HLA system is very polymorphic, a large number of specific primers are required to obtain a low-resolution HLA type. PCR-SSP requires prior knowledge of the sequence to be detected and may not detect novel HLA types.

PCR Sequence-Specific Oligonucleotide Probing

In this technique, the gene of interest is amplified using primers designed to anneal with a common DNA sequence. Designed oligonucleotide probes then bind to specific HLA sequences of interest. This requires either immobilising the DNA to be typed to an inert support and hybridising with sequence-specific oligonucleotide probes, or

Table 4.3 Advantages and disadvantages of DNA-based techniques.

Technique	Advantages	Disadvantages
Sequence-specific oligonucleotide probing (SSOP)	Needs fewer pairs of genetic primers Fewer reactions to set up Larger number of samples can be processed simultaneously Requires small amount of DNA Inexpensive	Different temperatures required for each probe Probes can cross-react with different alleles Large numbers of probes required to identify specificity Difficult to interpret pattern of reactions
Sequence-specific priming (SSP)	Provides rapid typing with higher resolution than SSOP PCR amplifications are carried out at same time, temperature and conditions Fast and simple to read and interpret	Many sets of primers are needed to fulfil HLA type Not ideal for multiple samples simultaneously
Sequencing-based typing (SBT)	Does not require previous sequence data to identify new allele Can provide high-resolution typing	Requires expensive reagents and equipment as well as experienced technicians
Sequencing-based typing by NGS	Provides the highest level of resolution and unambiguous results Cost-effective for high throughput	Requires more training Requires expensive reagents and equipment Requires automation for high-throughput testing Requires complex algorithms for the analyses and provision of results

HR, high resolution; NGS, next-generation sequencing; PCR, polymerase chain reaction.

immobilising the probes on inert support (e.g. well-plate, polystyrene bead) and hybridising with the amplified DNA (reverse SSOP). Detection of hybridisation and analysis of reaction patterns is largely automated and more amenable to typing of large numbers of samples than SSP.

DNA Sequencing-Based Typing

Sanger sequencing requires the denaturation of the DNA to be analysed, providing a single-strand template. Sequencing primers, exon or locus specific, are then added and the DNA extension is performed by the addition of Taq polymerase in the presence of excess nucleotides. The sequencing mixture is divided into four tubes, each containing a specific dideoxyribonucleoside triphosphate (ddATP). When these are incorporated into the DNA synthesis, elongation is interrupted with chain-terminating inhibitors. In each reaction, there is random incorporation of the chain terminators and therefore products of all sizes are generated. The sequencing products are detected by labelling the nucleotide chain inhibitors with radioisotopes or fluorescent dyes. The products of the four reactions are then analysed by electrophoresis in parallel lanes of a polyacrylamide–urea gel, and the sequence is read by combining the results of each lane using an automated DNA sequencer. Other methods combine all reactions utilising ddATP labelled with different fluorescent dyes, resulting in synthesis products of various sizes. The resulting sequence is then determined by capillary electrophoresis.

High-throughput high-resolution HLA typing using massive parallel clonal sequencing strategies and NGS platforms is also available, producing typing results with few ambiguities. With expanded gene coverage, sequencing can span traditional exons, as well as introns and untranslated regions. There are several commercial NGS products available for clinical use, offering comparable results but with differing hands-on versus automated requirements and analytical software [7]. High-resolution typing for matching haematopoietic recipients and donors has long been established practice, as it has resulted in better patient outcomes [8]. Recently, the clinical utility of high-resolution typing in the setting of solid organ transplantation has also been highlighted [9].

Formation of HLA Antibodies

In addition to antigenic peptide presentation to T cells, HLA molecules permit immune recognition of 'self' versus 'non-self'. Anti-HLA antibodies are the product of immune system encounters with unfamiliar antigens via direct or indirect allorecognition by host T cells. In direct allorecognition, host T cells directly recognise non-self HLA molecules expressed by donor tissues (e.g. dendritic cells or endothelial cells). Indirect allorecognition requires host T cell recognition of donor-derived HLA class I and II antigenic peptides as presented by the host's own antigen-presenting cells.

HLA antibodies commonly form after pregnancy, transplant or blood transfusion, termed alloimmunisation. HLA antibodies against the fetus's paternally inherited antigens are generated by the mother in about 11% of first pregnancies, increasing in frequency up to 32% with subsequent pregnancies [10]. Antibodies produced secondary to transplantation, known as donor-specific antibodies (DSAs), and following transfusion are further discussed below. The affinity, avidity and class of the antibody produced will depend on the persistence and type of immunological challenge, as well as the immune status of the host. Additionally, the strength and detectable presence of HLA antibodies can wax and wane over time.

HLA Antibody Detection

Many techniques are available to detect circulating HLA antibodies in patient plasma or serum prior to transplant or transfusion.

Complement-dependent cytotoxicity (CDC)–based antibody studies and solid-phase testing are the most commonly discussed.

CDC/Microlymphocytotoxicity Assay

The CDC test, also known as the microlymphocytotoxicity assay, is a functional assay that requires the incubation of patient plasma or serum with a panel of HLA-typed lymphocytes. After complement is added, the samples are evaluated for cell lysis. The lysis pattern is cross-referenced with the reagent cell typings to identify antibody identity. The number of observed lysis reactions in the panel of cells is used to determine a panel-reactive antibody (PRA) percentage. The inverse of the PRA indicates the probability of identifying a compatible donor within the testing panel's reference population. While this is one of the original antibody detection approaches, only high-concentration complement-fixing antibody–antigen interactions that activate complement via the classical pathway are identified. The CDC assay does not discriminate between HLA and non-HLA cytotoxic lymphocyte-reactive antibodies and generally detects only high-titre HLA antibodies, thus it does not identify all clinically relevant HLA antibodies.

Solid-Phase Antibody Detection Assays

Solid-phase approaches to HLA antibody analysis include enzyme-linked immunosorbent assay (ELISA) and flow cytometry–based bead testing, which are sensitive enough to identify clinically relevant antibodies not delineated by CDC evaluation. Both ELISA and flow-based bead testing involve HLA antibody detection using purified or recombinant HLA antigens bound to a solid platform. Following the addition of patient serum or plasma and wash steps, antibody binding is detected via secondary antihuman immunoglobulin. These techniques are HLA specific and detect both complement-fixing and non-complement-fixing antibodies. Depending on the assay set-up, the results may indicate the presence or absence of class I or II HLA antibodies or delineate antibody specificity.

Luminex technology, which uses multiplexed wells of fluorescently labelled beads analysed by dual-laser flow cytometry–based instruments, is the standard modality for HLA antibody evaluation in modern labs. Patient plasma or serum is incubated with fluorochrome-dyed polystyrene beads coated with specific purified or recombinant HLA antigens. The reaction is then washed to remove unbound antibodies. The beads are exposed to fluorophore-conjugated antihuman immunoglobulin (Ig)G (Fc-specific) antibody, which attaches to any bead-bound HLA antibody. The sample can then be fed through an analyser to detect both the unique internal fluorescence of the bead and the fluorophore-labelled antihuman IgG. The resulting data can indicate the presence and intensity of antibodies bound to each bead's attached HLA antigens, providing both sensitive and semi-quantitative output. The results of a single antigen bead assay can be input as 'unacceptable antigens' into a computer algorithm to define a calculated PRA (cPRA) percentage. An online cPRA calculator can be seen at https://optn.transplant.hrsa.gov/resources/allocation-calculators/cpra-calculator [11]. Additionally, allele-level antibody information provided by this assay has allowed for a new age of virtual crossmatching, further discussed below. Drawbacks of solid-phase testing include prozone effects, the inability to delineate complement-binding from non-complement-binding antibodies, and false positive reactions secondary to denatured or misfolded antigens binding clinically irrelevant antibodies.

Crossmatching for Compatibility Evaluation

Crossmatching for HLA purposes may occur prospectively and/or retrospectively using either physical or virtual means. The

fundamental role of the crossmatch is to determine recipient antibody compatibility with donor typing.

Physical Crossmatches

Both prospective and retrospective physical crossmatches (PXM) occur *in vitro*, utilising CDC or flow cytometric testing methods. A negative PXM result should indicate that no HLA antibodies of clinical significance (DSAs) are present in the patient's serum or plasma that may bind the donor's lymphocytes. A positive result suggests that DSAs are present. As with any test, there are many confounding factors that may alter crossmatch interpretation.

The complement-dependent cytotoxicity (CDC) crossmatch is like the CDC antibody testing described above, except that potential donor lymphocytes, rather than a panel of typed lymphocytes, are incubated with recipient plasma or serum. Complement is added and the sample evaluated for cell lysis. Only high-titre antibodies that activate complement via the classical pathway are identified. The CDC assay does not discriminate between HLA and non-HLA cytotoxic lymphocyte-reactive antibodies such as IgM autoantibodies. A positive CDC result is considered a contraindication to solid organ or bone marrow transplant, as it correlates to hyperacute rejection and graft loss.

In flow cytometric crossmatching, donor lymphocytes are mixed with patient sera, a secondary fluorescent reagent antibody is added to mark lymphocyte-bound antibody, and the mix is passed through a laser beam to quantify cellular fluorescence. Different cell populations can be identified by morphology/granularity and fluorescence. Reagent antibodies to CD3 or CD19 can identify T- or B-cell identity. Flow cytometric techniques are a sensitive indicator of DSAs; however, non-HLA lymphocyte-reactive antibodies of unclear clinical relevance may also be detected.

Virtual Crossmatches

Virtual crossmatches (VXM) occur via computer analysis. In the absence of a recent sensitising event, a patient's most current HLA antibody profile (as evaluated by solid-phase testing) can be compared with the potential donor's molecular HLA type to predict compatibility. Use of prospective VXM has facilitated reduced time to transplant, decreased ischaemia time of donor organs, as well as decreased time and mortality on the waiting list for candidate recipients. Given the sensitivity of solid-phase HLA antibody testing and molecular HLA typing, the VXM is highly predictive of PXM results. Virtual crossmatch is highly dependent on antibody analysis technique. There is significant variation in programmatic thresholds for antibody clinical significance, as well as the incorporation of complementary testing modalities (e.g. C1q and IgG subtype) and epitope and eplet analysis in the interpretation process [12]. A negative VXM result should indicate that no significant DSAs are present in the patient's serum or plasma. A positive result means that DSAs were detected in a recent sample that correspond to a prospective donor's molecular type. VXM is used as the primary method for pretransplant donor–recipient evaluation by many labs, regardless of the cPRA. Despite the data on VXM effectiveness in the highly alloimmunised, some programmes remain more conservative, still relying on prospective PXM, especially for patients who are allosensitised [13].

HLA and Solid Organ Transplantation

After ABO compatibility and organ size considerations, the avoidance of circulating HLA antibodies directed against a potential donor's antigens (DSA) is a critical determinant in donor–recipient pairing for most solid organ transplant programmes. The screening for and identification of significant HLA antibodies are performed prior to patient registration on a transplant waiting list. This registry ensures that predictably

incompatible donors are not considered for specific recipients during the organ allocation process. This antibody avoidance strategy is regularly employed for kidney, pancreas, lung, heart and small bowel donor selection, given data that correlate DSA with decreased graft survival. Furthermore, the appearance of *de novo* or increasing levels of pre-existing DSA after transplantation has been associated with graft rejection, indicating the importance of posttransplant antibody monitoring. Interestingly, donor–recipient HLA type matching is less consequential to transplant outcomes and is not routinely considered for organ acceptance.

Liver transplant remains a notable exception to the standard HLA antibody avoidance and posttransplant monitoring approaches used by other solid organ programmes. The hepatic organ appears relatively resistant to both preformed and *de novo* HLA antibodies, though data on outcomes with *de novo* class II DSA suggest the immunological protection may not be absolute. The role of DSA in liver graft survival remains an area of research and posttransplant DSA monitoring has become more common.

HLA and Haematopoietic Stem Cell Transplantation

In comparison to solid organ transplant considerations, HLA antigen matching is the predominant factor in stem cell transplant donor selection, followed by antibody avoidance and ABO considerations. Stem cell transplant recipients and donors should be typed at high resolution for HLA-A, -B, -C, -DRB1 and -DPB1. Additional typing of HLA-DQB1, -DRB3/4/5, -DQA1 and -DPA1 may also be helpful. For bone marrow (BM) or peripheral blood haematopoietic progenitor cell (HPC) sources, HLA matching at HLA-A, -B, -C and -DRB1 is recommended. Given that siblings have a 25% chance of being HLA identical, brothers and sisters of the patient are often the preferred donor candidates for a matched related donor transplant. When choosing between multiple HLA-matched options, younger age is the only donor characteristic associated with improved posttransplant overall survival. Haploidentical related donors may also be considered given both first-degree and second-degree relatives may be eligible. Selection preference in haploidentical transplant is similarly for younger donors to reduce graft-versus-host disease and improve survival outcomes [14].

If a fully matched or haploidentical related donor is unavailable, mismatched unrelated donors can be considered. Mismatching at any HLA-A, -B, -C or -DRB1 locus is associated with decreased survival. No locus mismatch is better tolerated than others, apart from rare 'permissive mismatches' such as HLA-C*03:03 versus HLA-C*03:04. Cases with these do not result in allorecognition and have comparable outcomes to 8/8 HLA-matched donors [14].

If a mismatch between donor and recipient typing is known at any class I or II locus, HLA antibody testing of the recipient is warranted. If DSA is present, a crossmatch may be used to determine compatibility. Transplanting against preformed DSA is not recommended and is associated with primary graft failure [14]. In the absence of alternative donors, a desensitisation plan can reduce the recipient's circulating HLA antibodies prior to transplant.

Umbilical cord blood (UCB) units are another stem cell source with unique considerations. High-resolution HLA-A, -B, -C and -DRB1 typing is recommended, but -DQB1 and -DPB1 typing may be helpful. HLA mismatches in UCB are also associated with negative outcomes, but available cell dose and unit quality considerations supersede matching for donor selection. Low cell doses per kilogram of recipient body weight are associated with slower and impaired engraftment and decreased overall survival, limiting the utility of small-volume UCB sources in adult recipients. The use of multiple units and *ex vivo* cellular expansion may eventually overcome this cell dose limitation. Additional considerations

for this donor cell source include unit age, RBC content and cord blood banking location, accreditation and licensure [14].

HLA and Blood Transfusion

HLA class I- and II-bearing white cells and class I-expressing platelets, as well as soluble HLA antigens and antibodies, may be present in transfused products. Immature nucleated and mature RBCs may also express class I HLA antigens known as Bennett-Goodspeed (Bg) antigens (e.g. HLA-A2, -A68, -A69, -B7, -B57, -B58) capable of alloimmunisation [15]. Transfused non-self HLA antigens and antibodies can activate T cells and lead to the development of antibodies and/or effector cells responsible for some of the serious complications of blood transfusion:

- Febrile non-haemolytic transfusion reactions (FNHTR).
- Transfusion-related lung injury (TRALI).
- Transfusion-associated graft-versus-host disease (TA-GVHD).
- Immune refractoriness to platelet transfusions.

Prestorage leucocyte reduction of blood products has been shown to reduce but not eliminate these reactions and alloimmunisation incidence in multi-transfused patients [16,17].

HLA and Disease Association

Certain HLA genes have been associated with a variety of diseases (Table 4.4). These associations are typically incomplete secondary to

Table 4.4 HLA type and associated diseases [18,19].

HLA antigen or allele	Associated disease	Notes
A29	Birdshot chorioretinopathy	Chronic posterior uveitis that results in posterior eye segment inflammation and retinal atrophy Virtually all patients of this rare disease carry this allele, which is frequent in European populations
B27	Ankylosing spondylitis	Chronic inflammatory disease involving the axial skeleton and sacroiliac joints Autoimmune process secondary to arthritogenic peptides presentation
B51	Behçet's disease	Vasculitis of the orogential mucosa, skin and eyes Predominantly affects those from Asian, Middle Eastern or Mediterranean ancestry
C6	Psoriasis	Chronic inflammatory and hyperproliferative skin disorder Specific alleles used to predict treatment responses to varied monoclonal antibodies
DQA1*05 DQB1*02 DQB1*03:02	Coeliac disease	Gluten-sensitive autoimmune enteropathy strongly associated with HLA-DQ2 (encoded by DQA1*05 and DQB1*02) and DQ8 (encoded by DQB1*03:02)
A*02:01 DQ2 DQ8 DR4	Type I diabetes	Chronic condition in which the pancreas produces little or no insulin Increased genetic risk

(*Continued*)

Table 4.4 (Continued)

HLA antigen or allele	Associated disease	Notes
DQB1*06:02	Narcolepsy	Chronic hypersomnia disorder due to autoimmune destruction of hypocretin-producing cells DQB1*06:03 and 06:01 are protective
DR4	Rheumatoid arthritis	Chronic inflammatory disorder of the joints Increased genetic risk
DR4 DR15	Multiple sclerosis	Demyelinating disease of the brain and spinal cord Increased genetic risk
DR15	Anti-glomerular basement membrane (Goodpasture) disease	Autoimmune disease of the lungs and kidney basement membranes Increased genetic risk DR1 is protective

variable penetrance, gene interactions and environmental factors. Mechanisms of disease association include LD with the relevant disease susceptibility gene, preferential presentation of pathogenic peptides by relevant HLA molecules, and molecular mimicry of host-derived peptides by pathogenic peptides [18].

HLA and Pharmacogenomics

Just as HLA types have been associated with a myriad of autoimmune and chronic inflammatory disorders, HLA alleles have also been linked to severe drug hypersensitivity reactions such as Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (Table 4.5).

Table 4.5 HLA type and associated pharmacological concerns [19].

HLA allele	Drug-associated complication/notes
HLA-B*57:01	Taking abacavir (antiretroviral) may result in a life-threatening hypersensitivity reaction with multi-organ involvement Prospective HLA genotyping is required by the US Food and Drug Administration (FDA)
HLA-B*15:02	Taking carbamazepine (antiepileptic) may result in SJS/TEN, particularly in those of Asian descent Prospective HLA genotyping is required by the FDA
HLA-B*58:01	Taking allopurinol (uric acid reducer) may result in SJS/TEN, particularly in Asian and African American populations Prospective HLA genotyping is recommended by the Clinical Pharmacogenetics Implementation Consortium and by the American College of Rheumatology

SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

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KEY POINTS

1) HLA genes are highly polymorphic and exhibit substantial linkage disequilibrium.
2) HLA types can be defined using DNA-based techniques at various degrees of resolution depending on the clinical need and relevance.
3) Multiple techniques are used to define HLA specificity and compatibility, including cytotoxic, solid phase, flow cytometric and virtual evaluation.
4) HLA antibodies can be produced following pregnancy, transplantation and transfusion.
5) Solid organ transplant programmes, with the exception of liver, routinely use HLA antibody avoidance strategies to pair waitlisted recipients with available organs.
6) In the stem cell transplantation setting, HLA matching for HLA class I and II types is critical to donor selection, followed by HLA antibody avoidance.
7) HLA molecules are crucial in the induction and regulation of immune responses and in the outcome of transplantation using allogeneic-related and -unrelated donors, and are also responsible for some of the serious immunological complications of blood transfusion.
8) HLA alleles are associated with autoimmune and inflammatory diseases, as well as adverse drug reactions.

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5 Platelet and Neutrophil Antigens

Brian R. Curtis

Platelet and Neutrophil Immunology Laboratory and Blood Research Institute, Versiti, Milwaukee, WI, USA

Antigens on Platelets and Neutrophils

Antigens on human platelets and neutrophils are expressed on various membrane glycoproteins (GPs). Some of these GPs are only or primarily expressed on platelets or neutrophils and others are shared by multiple cell types (Table 5.1).

- Glycoproteins only or predominantly expressed by platelets or neutrophils:
  - Platelets: GPIIb, GPIb/IX/V.
  - Neutrophils: FcγRIIIb (CD16b), CD177.
- Glycoproteins expressed by multiple cell lineages:
  - Platelets and neutrophils: human leucocyte antigen (HLA) class I (A, B).
  - Platelets: GPIIIa, GPIa/IIa, GPIV, A, B, H, etc.

These antigens can be targeted by some or all of the following types of antibodies:

- Autoantibodies.
- Alloantibodies.
- Isoantibodies.
- Drug-dependent antibodies.

This chapter is divided into two sections: the first focuses on proteins expressed predominantly on platelets, and in particular the human platelet alloantigens (HPAs), while the second section focuses on the equivalent proteins and alloantigens expressed predominantly on neutrophils (human neutrophil antigens, HNAs). Salient points concerning the clinical significance of antibodies produced against HPAs and HNAs are also addressed.

Human Platelet Antigens

To date, 35 platelet antigens have been described (Table 5.2); almost all were first discovered during investigation of cases of fetal and neonatal alloimmune thrombocytopenia (FNAIT). The majority of these antigens are located on the GPIIIa subunit of the GPIIb/IIIa integrin (αIIb/β3, CD41/CD61), which is present at high density on the platelet membrane and seems to be particularly polymorphic and immunogenic (Figure 5.1). Others are located on the GPIIb (CD41) subunit, on the GPIa subunit of GPIa/IIa (CD49b/CD29), GPIb/IX/V (CD42b/CD42a/CD42d) and CD109 [1].

These receptor complexes are critical to platelet function and are responsible for the stepwise process of platelet attachment to the damaged vessel wall, activation, aggregation and clot formation. GPIb/IX/V is the receptor for von Willebrand factor (vWF) and is implicated in the initial tethering of platelets to damaged endothelium. GPIbα bound to vWF interacts with collagen, facilitating

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Table 5.1 Antigen expression on peripheral blood cells.

Antigen	Erythrocytes	Platelets	Neutrophils	B lymphocytes	T lymphocytes	Monocytes
A, B, H	+++	+/+++[*]	–	–	–	–
I	+++	++	++	–	–	–
Rh[**]	+++	–	–	–	–	–
K	+++	–	–	–	–	–
HLA class I	–/(+)	+++	++	+++	+++	+++
HLA class II	–	–	–/+++[†]	+++	–/+++[†]	+++
GPIIb/IIIa	–	+++	(+)[‡]	–	–	–
GPIa/IIa	–	++	–	–	++	–
GPIb/IX/V	–	+++	–	–	–	–
CD109	–	(+)/+[†]	–	–	–/++[†]	(+)
FcγRIIIb (CD16b)	–	–	+++	–	–	–
CD177	–	–	+++[§]	–	–[††]	–
CTL-2	–	(+)	++	++	+++	?/–
CD11b/18	–	[†]	++	++	++	++[ɣ]
CD11a/18	–	[†]	++	++	++	++
CD36 (GPIV)	–/+∫	+++	-	-	-	+++

+++, ++, + Level of antigen expression in decreasing order; (+) weak expression; ? not known.
[*] Platelets from 4–6% of individuals have 'high expression' of A and/or B antigens.
[**] Non-glycosylated.
[†] On activated cells.
[‡] GPIIIa in association with an alternative α chain (αv).
[§] Expressed on a subpopulation of neutrophils.
[ɣ] Also expressed on natural killer cells.
∫ On nucleated erythrocytes.
[††] Expressed on activated regulatory T (Treg) cells

Table 5.2 Human platelet antigens.

Antigens	Phenotypic frequency*	Glycoprotein	Amino acid change	Encoding gene	Nucleotide change	dbSNP number
HPA-1a HPA-1b	72% a/a 26% a/b 2% b/b	GPIIIa	Leu33Pro	*ITGB3*	176T > C	rs5918
HPA-2a HPA-2b	85% a/a 14% a/b 1% b/b	GPIbα	Thrl45Met	*GPIBA*	482C>T	rs6065
HPA-3a HPA-3b	37% a/a 48% a/b 15% b/b	GPIIb	Ile843Ser	*ITGA2B*	2621T >G	rs5911
HPA-4a HPA-4b	>99.9% a/a <0.1% a/b <0.1% b/b	GPIIIa	Argl43Gln	*ITGB3*	506G > A	rs5917
HPA-5a HPA-5b	88% a/a 20% a/b 1% b/b	GPIa	Glu505Lys	*ITGA2*	1600G >A	rsl0471371
HPA-6b	<1%	GPIIIa	Arg489Gln	*ITGB3*	1544G > A	rsl3306487
HPA-7b	<0.1%	GPIIIa	Pro407Ala	*ITGB3*	1297C> G	rs121918448
HPA-8b	<0.1%	GPIIIa	Arg636Cys	*ITGB3*	1984C> T	rs151219882
HPA-9b	<0.1%	GPIIb	Val837Met	*ITGA2B*	2602G > A	rs74988902
HPA-10b	<0.1%	GPIIIa	Arg62Gln	*ITGB3*	263G > A	rs200358667
HPA-11b	<0.1%	GPIIIa	Arg633His	*ITGB3*	1976G >A	rs377302275
HPA-12b	<0.1%	GPIbβ	Glyl5Glu	*GPIBB*	119G > A	rs375285857
HPA-13b	<0.1%	GPIa	Met799Thr	*ITGA2*	2483C > T	rs79932422
HPA-14b	<0.1%	GPIIIa	Lys611del	*ITGB3*	1909_1911del^{AAG}	NA
HPA-15a HPA-15b	35% a/a 42% a/b 23% b/b	CD109	Ser682Tyr	*CD109*	2108C> A	rsl0455097

(*Continued*)

Table 5.2 (Continued)

Antigens	Phenotypic frequency*	Glycoprotein	Amino acid change	Encoding gene	Nucleotide change	dbSNP number
HPA-16b	<0.1%	GPIIIa	Thrl40Ile	*ITGB3*	497C > T	rs74708909
HPA-17b	<0.1%	GPIIIa	Thrl95Met	*ITGB3*	662C > T	rs770992614
HPA-18b	<0.1%	GPIa	Gln716His	*ITGA2*	2235G>T	rs267606593
HPA-19b	<0.1%	GPIIIa	Lysl37Gln	*ITGB3*	487A>C	rs80115510
HPA-20b	<0.1%	GPIIb	Thr619Met	*ITGA2B*	1949C>T	rs78299130
HPA-21b	<0.1%	GPIIIa	Glu628Lys	*ITGB3*	1960G >A	rs70940817
HPA-22b	<0.1%	GPIIb	Lysl64Thr	*ITGA2B*	584A>C	rsl42811900
HPA-23b	<0.1%	GPIIIa	Arg622Trp	*ITGB3*	1942C > T	rsl39166528
HPA-24b	<0.1%	GPIIb	Ser472Asn	*ITGA2B*	1508G > A	rs281864910
HPA-25b	<0.1%	GPIa	Thrl87Met	*ITGA2*	3347C> T	rs771035051
HPA-26b	<0.1%	GPIIIa	Lys580Asn	*ITGB3*	1818G>T	rs1156382155
HPA-27b	<0.1%	GPIIb	Leu841Met	*ITGA2B*	2614C> A	rsl49468422
HPA-28b	<0.1%	GPIIb	Val740Leu	*ITGA2B*	2311G>T	rs368953599
HPA-29b	<0.1%	GPIIIa	Thr7Met	*ITGB3*	98C> T	rs544276300
HPA-30b	<0.1%	GPIIb	Gln806His	*ITGA2B*	2511G>C	rs377753373
HPA-31b	<0.1%	GPIX	Pro123Leu	*GP9*	368C>T	rs202229101
HPA-32b	<0.1%	GPIIIa	Asn174Ser	*ITGB3*	521A>G	rs879083862
HPA-33b	<0.1%	GPIIIa	Asp458Gly	*ITGB3*	1373A>G	rs1555572829
HPA-34b	<0.1%	GPIIIa	Arg91Trp	*ITGB3*	349C>T	rs777748046
HPA-35b	<0.1%	GPIIIa	Arg479His	*ITGB3*	1514A>G	rs779974422

The current table can be viewed at https://www.versiti.org/hpa
*Based on studies of Caucasians.
rs, international SNP reference number in dbSNP database; SNV, single nucleotide variant.

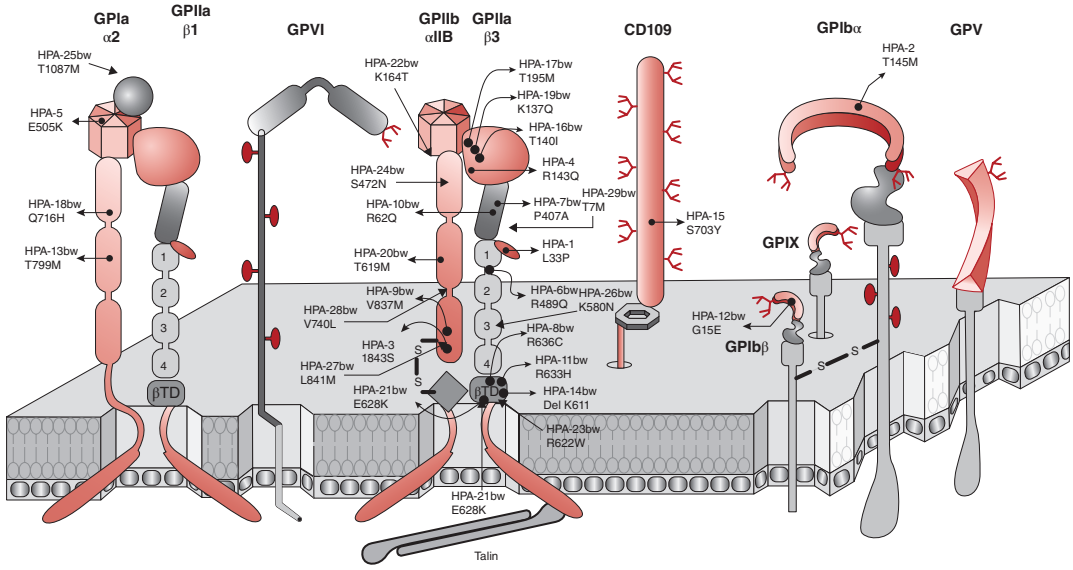

Figure 5.1 Representation of the platelet membrane and the glycoproteins (GP) on which the human platelet antigens (HPAs) are localised. From left to right are depicted GPIa/IIa, GPIIb/IIIa, CD109 and GPIb/IX/V. The molecular basis of the HPAs is indicated by black dots, with the amino acid change in single-letter code and by residue number in the mature protein.

the interaction of collagen with its signalling (GPVI) and attachment receptors (GPIa/IIa). Outside-in signalling via GPVI leads to conformational changes in integrins GPIIb/IIIa and GPIa/IIa from 'locked' to 'open' configurations, exposing the high-affinity binding sites for fibrinogen and collagen, respectively. GPIIb/IIIa is the major platelet fibrinogen receptor and is critical to the final phase of platelet aggregation, but it also binds fibronectin, vitronectin and vWF. The function of CD109 has not been fully elucidated, although recent studies suggest a role in regulation of transforming growth factor (TGF)-β–mediated signalling. Glanzmann's thrombasthenia and Bernard–Soulier syndrome are rare and severe, autosomal-recessive bleeding disorders caused by platelet dysfunction due to deletions or mutations in the genes encoding GPIIb and GPIIIa, or GPIbα, GPIbβ and GPIX, respectively [2].

Inheritance and Nomenclature

Most HPAs have been shown to be biallelic, with each allele being co-dominant, although recently the HPA-1, -5 and -7 systems have been shown to have third alleles. The nomenclature for HPAs involves consecutive numbering (HPA-1, -2, -3 and so on; see Table 5.2) according to the date of discovery, with the major allele in each system designated 'a' and the minor allele 'b' [3]. Antigens are only included in a system if antibodies against the alloantigen have been reported.

For all but one of the 35 HPAs, the difference between the two alleles is a single nucleotide variant (SNV), which changes the amino acid in the corresponding protein (Figure 5.2). Six HPAs are grouped into biallelic HPA systems (HPA-1–5 and HPA-15) and for all of these, except HPA-3 and HPA-15, the minor allele frequency is ≤ 0.1% in white populations. Homozygosity for the minor allele is therefore relatively rare, so providing compatible blood components for patients with antibodies against high-frequency HPA antigens is very difficult. Some HPAs are population specific, for example the frequency of HPA-1b is ~2% in white populations but absent in populations of the Far East; conversely, HPA-4b is not present in whites but is present in Far Eastern and Hispanic and Latino populations. It is therefore important to take population frequency into account when investigating clinical cases of suspected HPA alloimmunisation.

Typing for HPAs

Many DNA-based typing techniques have been developed to determine HPA genotypes [4,5]. One such assay is the polymerase chain reaction using sequence-specific primers (PCR-SSP). This is a fast and reliable technique and has become a cornerstone in platelet immunology laboratories. High-throughput HPA SNV typing techniques with automated readout, such as endpoint genotyping assays and next-generation sequencing, are now also in routine use and allow rapid, high-throughput genotyping that is especially useful for routine donor typing in blood centres.

Genotyping of fetal DNA from amniocytes or chorionic villus biopsy samples is of clinical value in cases of HPA alloimmunisation in pregnancies where there is a history of severe FNAIT and the father is heterozygous for the implicated HPA. Non-invasive HPA genotyping assays based on the presence of trace amounts of fetal DNA in maternal plasma have been recently described and reduce the risk to the fetus from invasive sampling procedures [6].

Platelet Isoantigens, Autoantigens and Drug-Dependent Antigens

GPIV/CD36 is absent from the platelets of 2–8% of individuals of black African descent and 3–10% of Japanese and other Eastern Asian and Middle Eastern populations [7]. Approximately 1% of these individuals also do not express CD36 on their monocytes, and if these individuals are exposed to GPIV-positive

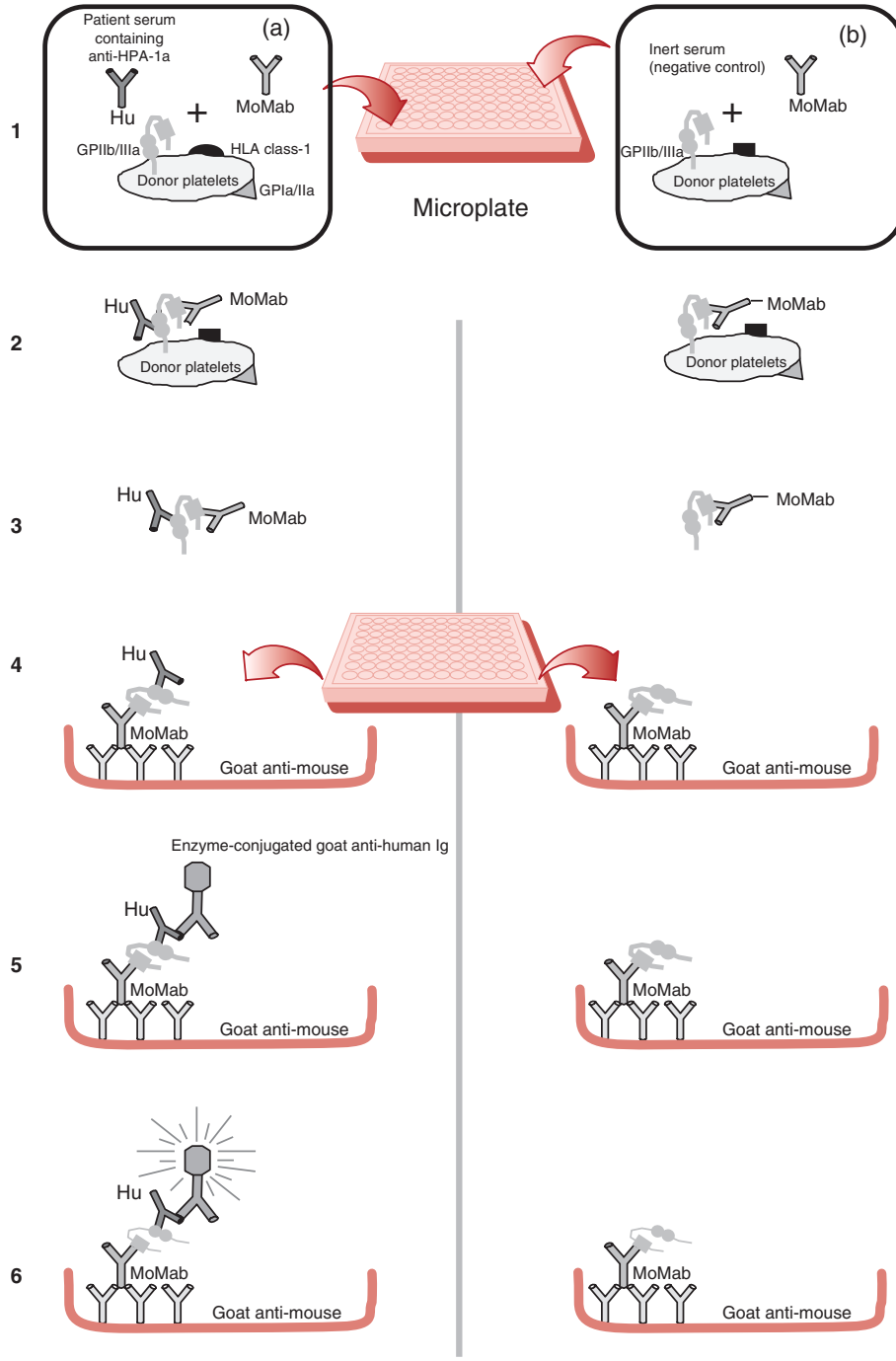

Figure 5.2 Monoclonal antibody immobilisation of platelet antigens (MAIPA) assay. (1) Human (Hu) serum and murine monoclonal antibody (MoMab) directed against glycoprotein being studied, e.g. GPIIb/IIIa, are sequentially incubated with target platelets: in (a) the test serum contains anti-HPA-1a and in (b) no platelet antibodies are present. (2) After incubation, a trimeric (a) or dimeric (b) complex is formed. Excess serum antibody and MoMab are removed by washing. (3) The platelet membrane is solubilised in non-ionic detergent, releasing the complexes into the fluid phase; particulate matter is removed by centrifugation. (4) The lysates containing the glycoprotein/antibody complexes are added to wells of a microtitre plate previously coated with goat antimouse antibody. (5) Unbound lysate is removed by washing and enzyme-conjugated goat antihuman antibody is added. (6) Excess conjugate is removed by washing and substrate solution is added. Cleavage of substrate, i.e. a colour reaction, indicates binding of human antibody to target platelets.

blood through pregnancy or transfusion, they may produce GPIV isoantibodies. Such antibodies can cause FNAIT, posttransfusion purpura or platelet refractoriness, and may be responsible for febrile non-haemolytic transfusion reactions (FNHTRs). Similarly, formation of isoantibodies can complicate both the pregnancies and transfusion support of patients with Glanzmann's thrombasthenia and Bernard–Soulier syndrome. Interestingly, GPIV-negative platelet transfusions are effective in raising platelet levels in GPIV-immunised patients [8].

GPs carrying the HPAs are also the targets of autoantibodies in immune thrombocytopenia (ITP); these autoantibodies bind to the platelets of all individuals, regardless of their HPA type. Platelet autoimmunity is frequently associated with B-cell malignancies and during immune cell re-engraftment following haemopoietic stem cell transplantation. In both situations, the presence of autoantibodies may contribute to the refractoriness to donor platelets.

Numerous drugs, and even some foods and herbal remedies, can associate with platelet GPs or preformed antibodies to form antigens that elicit the formation of drug-dependent antibodies (DDAbs) in certain patients [9]. DDAbs only bind to the GP in the presence of a drug; a classic example is quinine. Typically, quinine-dependent antibodies bind to GPIIb/IIIa and/or GPIb/IX/V, although other GPs are sometimes the target. Other drugs frequently associated with drug-induced thrombocytopenia (DITP) include vancomycin, sulfamethoxazole-trimethoprim, piperacillin, carbamazepine, rifampin and oxaliplatin. In haemato-oncology patients, who often receive a spectrum of drugs, unravelling the causes of persistent thrombocytopenia or poor responses to platelet transfusions can be complex because of the many possible causes of thrombocytopenia. Testing the patient's serum for the presence of DDAbs can help in determining whether the thrombocytopenia is drug induced. If the thrombocytopenia is drug mediated, withdrawal of the drug will result in recovery of the platelet count once the drug is eliminated from the circulation.

Another form of drug-dependent thrombocytopenia may be observed in coronary artery disease patients treated with ReoPro (abciximab). This function-blocking chimeric human–mouse F(ab) fragment against GPIIb/IIIa causes precipitous thrombocytopenia in approximately 1% of patients due to the presence of pre-existing antibodies against ReoPro-induced neoepitopes.

The interaction of heparin and other polyanions with platelet factor 4 induces epitope formation that can cause antibody production and lead to heparin-induced thrombocytopenia (HIT; see Chapter 16), but the reduction in platelet count is less profound than in classic examples of drug-mediated immune thrombocytopenia. The risk of thrombotic complications is the main concern in patients with HIT who show a mild but significant reduction in their platelet count after heparin administration.

Detection of HPA Alloantibodies

Optimal testing for platelet antibodies requires use of multiple methods. The platelet immunofluorescence test (PIFT) by flow cytometry is highly sensitive, but is unable to distinguish between HPA and HLA class I antibodies. Despite this limitation, it remains widely used as a whole-cell assay capable of detecting a wide range of antibody specificities, and is especially useful in detecting both autoantibodies and alloantibodies. Assays that use purified or captured GPs, such as the monoclonal antibody immobilisation of platelet antigens (MAIPA) and platelet antibody bead array (PABA) assays, were developed for the detection and identification of HPA antibodies without interference by HLA class I antibodies [10].

The widely used MAIPA assay captures specific GPs using monoclonal antibodies bound to a microplate and can be used to analyse complex mixtures of platelet antibodies in patient sera [10]. The principle of this assay is shown in Figure 5.2. The MAIPA

assay requires considerable operator expertise in order to ensure maximum sensitivity and specificity, and the selection of appropriate screening cells is critical. Use of monoclonal antibodies that block binding of the patient's HPA antibodies, and platelets heterozygous for the relevant HPA or from donors who have a low expression of particular antigens, e.g. HPA-15, may result in failure to detec clinically significant alloantibodies. Furthermore, evidence suggests that integrin conformation is an important factor in assay sensitivity. Recently, monoclonal antigen capture assays similar to MAIPA that use individual beads coated with GP-specific monoclonal antibodies, like PABA, have been developed. These assays are highly sensitive and allow for multiplexing for simultaneous detection of antibodies against multiple HPAs [11].

Clinical Significance of HPA Alloantibodies

HPA alloantibodies are responsible for the following clinical conditions:

- FNAIT: this condition is described in detail below (but also see Chapter 35).
- Refractoriness to platelet transfusions (described in detail in Chapter 32).
- Posttransfusion purpura (described in detail in Chapter 15).

Fetal and Neonatal Alloimmune Thrombocytopenia

History

FNAIT is a well-recognised clinical entity and the platelet counterpart of haemolytic disease of the fetus and newborn (HDFN), with an estimated incidence of severe thrombocytopenia due to maternal HPA antibodies of 1 per 1000–1200 live births [12]. Unlike HDFN, about 12–30% of FNAIT cases occur in first pregnancies.

Definition and Pathophysiology

FNAIT is due to maternal HPA alloimmunisation caused by fetomaternal incompatibility for a fetal HPA inherited from the father that is absent in the mother. Maternal IgG alloantibodies against the fetal HPA cross the placenta, bind to fetal platelets and may reduce platelet survival. Severe thrombocytopenia in the term neonate, accompanied by haemorrhage, is generally caused by HPA-1a antibodies most frequently observed if the mother is white. Antibodies against HPA-2 and HPA-4 antigens are more often implicated in cases of Far Eastern ethnicity. In the latter, and in black Africans and Middle Eastern groups, GPIV deficiency should also be considered. Antibodies against HPA-5b and -5a tend to cause less severe FNAIT than anti-HPA-1a, probably due to the low copy number of the GPIa/IIa complex (3000/platelet versus 80 000/platelet for GPIIb/IIIa).

FNAIT due to alloantibodies against other HPAs is infrequent, and HLA class I antibodies, present in 15–35% of multiparous women, are rarely, if ever, the cause of FNAIT. Clearance of IgG-coated fetal platelets takes place predominantly in the spleen through interaction with mononuclear cells bearing Fcγ receptors for the constant domain of IgG.

HPA-1a is known to be expressed on fetal platelets from 16 weeks' gestation and placental transfer of IgG antibodies can occur as early as 14 weeks, so thrombocytopenia can occur early in pregnancy and intracranial haemorrhage (ICH) has been reported as early as 16 weeks' gestation.

Incidence

Prospective screening of pregnant white women has shown that about 1 in 1200 neonates has severe thrombocytopenia ($<50 \times 10^9$/L) because of alloimmunisation against HPA-1a. However, the authors' experience and other studies, where prospective screening was not carried out [13], indicate that the number of samples referred for investigation of suspected FNAIT is considerably lower, which suggests that many cases are undiagnosed.

Clinical Features

A typical case of FNAIT presents with skin bleeding (purpura, petechiae and/or ecchymoses) or more serious haemorrhage, such as

ICH, in a full-term and otherwise healthy newborn with a normal coagulation screen and isolated thrombocytopenia. There are less common presentations of FNAIT *in utero*, including ICH, ventriculomegaly, cerebral cysts and hydrocephalus, which may be discovered by routine ultrasound. However, the authors' experience and other studies [14] demonstrate that the majority of such cases show negative maternal serology for HPA antibodies. Although rare, hydrops fetalis has been reported in association with FNAIT and this diagnosis should be considered if there are no other obvious reasons for the hydrops.

The precise incidence of ICH due to FNAIT is unknown, but conservative estimates suggest that it is as low as 1 in 10 000 live births. Nearly 50% of severe ICHs occur *in utero*, usually between 30 and 35 weeks' gestation, but sometimes before 20 weeks'. At the other end of the clinical spectrum, and more commonly, FNAIT is discovered incidentally when a blood count is performed for other reasons.

Differential Diagnosis

Other causes of neonatal thrombocytopenia are infection, prematurity, intrauterine growth retardation, inherited chromosomal abnormalities (particularly trisomy 21), maternal ITP and, very rarely, inherited forms of inadequate megakaryopoiesis. Maternal platelet autoimmunity is rarely associated with severe thrombocytopenia in the neonate, but should be considered in women with a history of ITP.

Laboratory Investigations

Only antibodies against HPAs or isoantibodies against GPIIb/IIIa, GPIb/IX, CD109 and GPIV are thought to cause the vast majority of alloimmune thrombocytopenia in the fetus and neonate, although there are reports of platelet autoantibodies from patients with ITP crossing the placenta and causing neonatal thrombocytopenia. There are also rare reports involving high-titre maternal IgG anti-A, -B targeting fetal platelets expressing elevated levels of A and/or B blood group antigens [15].

For appropriate clinical management, the cause of severe thrombocytopenia in an otherwise healthy neonate should be urgently investigated. Screening for maternal HPA antibodies must be carried out, preferably by an experienced reference lab using techniques with appropriate sensitivity and specificity. HPA antibodies are detected in approximately 30% of referrals of suspected FNAIT referred to the Platelet and Neutrophil Immunology Reference Laboratory, Milwaukee, WI, USA. The most frequently detected antibody specificities are HPA-1a and HPA-5b, which are implicated in about 85% and 10% of clinically diagnosed cases of FNAIT, respectively. The ability of an HPA-1a-negative mother to form anti-HPA-1a is significantly controlled by the class II HLA *DRB3*01:01* allele. This allele is present in approximately 30% of white women, and the chance of HPA-1a antibody formation is greatly enhanced in HPA-1a-negative women who are HLA *DRB3*01:01* positive compared to *DRB3*01:01*-negative women (odds ratio of 140). The absence of HLA *DRB3*01:01* has a negative predictive value of as high as 99% for HPA-1a alloimmunisation, but its positive predictive value is only 35%, limiting its potential usefulness as part of an antenatal screening programme. However, it remains of clinical use when counselling female siblings from index cases who have formed HPA-1a antibodies in pregnancy. About 12% of HPA-1a-negative women exposed to a HPA-1a-positive fetus develop anti-HPA-1a in pregnancy, and of these, about 30% will deliver a neonate with a platelet count $< 50 \times 10^9$/L.

Molecular typing of the parents and neonate for HPA-1, -2, -3, -5, -6, -9 and -15 should be performed, because the results will be informative when interpreting antibody investigation results. For patients from the Far East, HPA-4 must also be included, and the platelets should be investigated for GPIV expression status.

Alloimmunisation against low-frequency HPAs, e.g. HPA-9b, explains some FNAIT referrals that have a negative antibody screen for the common HPA antibody

specificities. A practical approach to detecting antibodies against low-frequency antigens that are absent from cells used in antibody screening panels is to perform a crossmatch of maternal serum against paternal platelets, and to genotype paternal or affected infants' DNA samples for low-frequency HPA when GP-specific antibodies are detected against paternal platelets only. It is also necessary to exclude positive findings due to ABO or HLA class I antibodies.

Neonatal Management

An umbilical cord platelet count of $< 100 \times 10^9$/L should be repeated using a venous sample and a blood film examined. The neonate should be examined for skin or mucosal bleeding if a low platelet count is confirmed. If the platelet count is $< 30 \times 10^9$/L or if there are signs of bleeding with a low count, strong consideration should be given to transfusing the neonate with donor platelets that are HPA-1a and -5b negative, as these will be compatible with the maternal HPA alloantibody in ≥ 95% of FNAIT cases. Alternatively, if the mother is able, washed maternal platelets can be used. If HPA-compatible platelets are not immediately available and there is an urgent clinical need for transfusion, then random, ABO and RhD-compatible donor platelets should be used [16]. In a typical case, the platelet count should recover to normal within a week, although a more protracted recovery can occur. Intravenous immunoglobulin (IvIgG) is not recommended as a first-line treatment as it is only effective in about 75% of cases and there is a delay of 24–48 hours before a satisfactory count is achieved; this is in contrast to the accelerated effect of transfusion of platelets. A cerebral ultrasound scan of the baby within the first week of life should be considered if the platelet count is $< 50 \times 10^9$/L and is recommended when the platelet count is $< 30 \times 10^9$/L.

Antenatal Management

In a subsequent pregnancy of a mother with serologically confirmed FNAIT, the clinical management needs to be planned by a team experienced in the management of the risks of this condition. Treatment during the subsequent pregnancy is based on the history of haemorrhage and fetal/neonatal thrombocytopenia in previous pregnancies.

Infusion of high-dose IvIgG to the mother is the safest and most effective intervention to reduce the risk of ICH in the at-risk fetus [17]. The dose is 1 g/kg bodyweight at weekly intervals, usually from 20 weeks' gestation onwards; some fetal medicine specialists will use a higher dose (2 g/kg/week) and/or between 12 and 20 weeks' gestation, depending on the history of FNAIT in previous pregnancies. Early commencement of treatment is indicated where there is a history of antenatal ICH in previous pregnancies, because the earliest reports of ICH are at 16 weeks. IvIgG administration has a 97.8% success rate for prevention of fetal ICH [18]. The delivery needs careful planning between obstetric, paediatric and haematology teams to ensure an appropriate mode of delivery, and close liaison with blood transfusion services for timely provision of HPA-compatible platelets for the neonate. For neonates who have been transfused *in utero*, irradiation of cellular blood components is recommended, as is the case for platelet transfusions from any first-degree relative.

Counselling

Counselling of couples with an index case about the risks of severe fetal/neonatal thrombocytopenia in a subsequent pregnancy needs to be based on disease severity in the infant(s) and outcome of immunological investigations. The following should be taken into account:

- Thrombocytopenia in subsequent cases is as severe or, generally, more severe.
- The best predictors of severe fetal thrombocytopenia in a future pregnancy are antenatal ICH and severe thrombocytopenia (platelet count $< 30 \times 10^9$/L) in a previous pregnancy.
- Antibody specificity.
- Antibody titre and bioactivity have been investigated to determine whether these

parameters have a predictive role in determining the severity of FNAIT – contradictory data have been obtained and currently are probably of no value in informing clinical management.

- HPA zygosity of the partner. If the father is heterozygous, there is a 50% chance a future fetus will inherit the implicated HPA and be at significant risk of developing FNAIT. In such cases, prenatal genotyping of the fetus should be performed.

Human Neutrophil Antigens

Neutrophils, like platelets, express their own unique cell surface antigens. There are common antigens that have a wider distribution on other blood cells and tissues, e.g. I and P blood group systems and HLA class I. Unlike erythrocytes and platelets, neutrophils do not express ABO antigens. There are 'shared' antigens that have a limited distribution among other cell types, such as HNA-4 and HNA-5 polymorphisms associated with CD11/18. There are also a limited number of truly neutrophil-specific antigens, such as HNA-1a, HNA-1b and HNA-1c on FcγRIIIb/CD16b. The current nomenclature for HNA includes antigens that are both cell specific and 'shared' (Table 5.3) [19].

HNA-1

The three antigens that comprise HNA-1 are localised on the neutrophil FcγRIIIb (CD16b), one of two low-affinity receptors (R) for the constant domain (Fc) of human IgG(γ) that are found on neutrophils. FcγRIIIb is a glycosylphosphatidylinositol (GPI)-anchored membrane GP, and there are normally 100 000–200 000 copies per neutrophil. Two amino acid differences in FcγRIIIb define the difference between HNA-1a and -1b, while a single amino acid substitution (alanine 78 asparagine) defines the HNA-1c polymorphism. The expression of HNA-1c is frequently associated with the presence of an additional FcγRIIIb gene and increased expression of FcγRIIIb. The expression of HNA-1 antigens varies with ethnicity, with HNA-1a being more common in Chinese and Japanese populations and HPA-1b more common in whites.

The FcγRIIIb 'Null' phenotype is rare and is based on a double deletion or mutation of the *FcγRIIIb* gene. A maternal deficiency of FcγRIIIb can cause immune neutropenia in the newborn due to maternal FcγRIIIb isoantibodies. The FcγRIIIb molecule can also be the antigenic target in autoimmune neutropenias. Of note is that as many as 23% of autoantibodies in autoimmune neutropenia of infancy have 'relative/stronger' reactivity for HNA-1a.

HNA-2

HNA-2, formerly known as HNA-2a or NB1, is localised on CD177 and expressed as a GPI-anchored membrane GP found both on the neutrophil surface membrane and on secondary granules. HNA-2 simply indicates expression of CD177 on neutrophils. Neutrophils from about 3% of individuals do not express CD177, and such individuals are capable of producing CD177 antibodies when exposed to the protein.

The percentage of neutrophils expressing CD177 varies between individuals and CD177 alloantibodies typically give a bimodal fluorescence profile with neutrophils from CD177-positive donors in immunofluorescence tests with a flow cytometric endpoint. CD177 antigen status can be determined by phenotyping with polyclonal or monoclonal antibodies or by genotyping *CD177* for c.787A>T, as recent evidence indicates that the majority of CD177-null individuals have c.787T [20].

HNA-3

HNA-3a and HNA-3b antigens are expressed on choline transporter-like protein 2 (CTL2), and in addition to neutrophils are also expressed on T and B lymphocytes and weakly on platelets. The polymorphism is determined by an SNV in the *SLC44A2* gene that results in

Table 5.3 Human neutrophil antigens.

Antigens	Frequency*	Glycoprotein	Amino acid change	Encoding gene	Nucleotide change
HNA-1a	54%	FcγRIIIb/	R36,L38,**N**65,A78,D82	*FCGR3B01*	108G,114C,194**A**,233C,244G
HNA-1b†	88%	CD16b	**S**36,L38,S65,A78,**N**82	*FCGR3B02*	108**C**,114T,194G,233C,244**A**
HNA-1c†	5%		S36,L38,S65,**D**78,N82	*FCGR3B03*	108C,114T,194G,233**A**,244A
HNA-2	97%	CD177	K293X	*CD177*	787A>T**
HNA-2 null	3%				
HNA-3a	94%	CTL2	Arg154Gln	*SLC44A2*	455G>A
HNA-3b	40%				
HNA-4a	99%	CD11b	Arg61His	*ITGAM*	230G>A
HNA-4b	1%				
HNA-5a	85%	CD11a	Arg766Thr	*ITGAL*	2372G>C
HNA-5b	54%				

* Frequencies based on studies of Caucasian/white populations. Significant base and amino acid differences in bold.
** Other variants have been reported that influence CD177 expression in rare cases.
† HNA-1b also carries the HNA-1d epitope, and HNA-1c also carries the HNA-1b epitope.

a A152G amino change in CTL2 that determines HNA-3a and HNA-3b, respectively. HNA-3b/b individuals can make HNA-3a antibodies, and 5–6% of whites and 16% of Han Chinese have the HNA-3b/b type. HNA-3a antibodies have been implicated in neonatal alloimmune neutropenia (NAIN) and cause particularly severe cases of transfusion-related acute lung injury (TRALI).

HNA-4 and HNA-5

The genes encoding the αM and αL subunits of the β_2 integrins CD11b/18 and CD11a/18 are polymorphic and are associated with HNA-4a/4b and HNA-5a/5bw, respectively. Alloantibody formation against these two polymorphisms has been observed in transfusion recipients, and recently cases of NAIN due to HNA-4a, HNA-4b and HNA-5a antibodies have been described. The low incidence of neonatal neutropenia associated with these antibodies is probably explained by the wide distribution of these proteins on granulocytes, monocytes and lymphocytes.

Detection of Neutrophil Antibodies

Reliable detection and identification of neutrophil antibodies are technically difficult due to the daily requirement for fresh, typed donor neutrophils, since neutrophils cannot be stored. The incidence of antibody-mediated neutropenia is comparatively rare and, therefore, the best strategy for investigation of clinical cases is a national reference laboratory where adequate technical expertise and reagents are available.

The granulocyte immunofluorescence test by flow cytometry (GIFT-FC) has the advantage of good sensitivity but lower specificity, i.e. it cannot readily distinguish between granulocyte-specific and HLA class I antibodies without further investigations. For some HNA systems, such as antigens expressed on CD16b, CD177 and CD11/18, the monoclonal antibody immobilisation of granulocyte antigens (MAIGA) assay can be applied to determine HNA specificity. The principles of the granulocyte immunofluorescence test and the MAIGA assay are analogous to the equivalent platelet tests (see Figure 5.2). Increased understanding of the molecular nature of HNAs expands the potential to develop recombinant HNAs, and both cell lines expressing recombinant proteins (rHNA) and soluble rHNA coupled to a solid phase have been described. These new assays have shown promise but, currently, generally lack the sensitivity and specificity of established techniques.

Like for HPA, HNA typing is performed by PCR-SSP or sequence-based typing techniques. However, HNA-2 is currently still commonly typed for on fresh neutrophils with CD177 monoclonal antibodies by GIFT-FC.

Clinical Significance of HNA Antibodies

Neutrophil-specific antibodies are implicated in:

- Neonatal alloimmune neutropenia (NAIN).
- Febrile non-haemolytic transfusion reactions (FNHTR).
- Transfusion-related acute lung injury (TRALI; see Chapter 11).
- Autoimmune neutropenia.
- Persistent post–bone marrow transplant neutropenia.

Neonatal Alloimmune Neutropenia

Maternal alloimmunisation against neutrophil-specific alloantigens on fetal/neonatal neutrophils is a condition analogous to FNAIT in terms of pathophysiology but, with an estimated incidence of 0.1–0.2% of live births, is comparatively rare as a clinically significant entity, although there are no reliable figures. Clinical presentation is mainly one of bacterial infections, with isolated neutropenia being the only haematological abnormality. The neutropenia may be severe, but is reversible, and newborn infants may require treatment with antibiotics and/or granulocyte colony-stimulating factor (G-CSF) to control bacterial infections and hasten recovery to a normal neutrophil count. The neutropenia in some cases has been reported to extend for up to 32 weeks. HNA-1a and -2 are the most commonly implicated antibody specificities, but all HNAs have been implicated.

FNHTR and TRALI

FNHTRs have a number of different causes. They can occasionally be associated with the presence of leucocyte (HLA and HNA) alloantibodies in the recipient. Serological investigations for platelet, HLA and granulocyte antibodies are of limited clinical value, as the diagnostic specificity of these tests for FNHTRs is low. Nonetheless, testing for HNA antibodies may be required in rare cases in which a severe FNHTR cannot be otherwise explained and washed components have proved ineffective.

TRALI is a severe and sometimes life-threatening transfusion reaction [20]. The majority of cases are caused by donor leucocyte alloantibodies against alloantigens present on the patient's leucocytes, although patient alloantibodies may be involved. HLA class I- and II-specific antibodies and HNA antibodies have been implicated as causal agents, with HNA-1a and HNA-3a antibody specificities being found most commonly [22]. TRALI investigations are logistically complex because of the need to contact all the implicated donors to obtain fresh blood samples. The donor samples are screened for both HLA and HNA alloantibodies. If antibodies are found, it is necessary to type the patient to determine whether they have the cognate antigen, and to type the donor to establish that they lack the antigen. In some cases, it may be necessary to screen a recipient's serum for antibodies or to perform a crossmatch between donor sera and the patient's granulocytes and lymphocytes.

Many blood transfusion services have taken steps to reduce the incidence of TRALI, for example by preventing females who have been pregnant from donating plasma and platelet components, and more recently by screening female donors with a pregnancy history for HLA and HNA antibodies. The success of these strategies has been demonstrated by the reduced incidence of TRALI in haemovigilance schemes [23].

Autoimmune Neutropenia

Autoimmune neutropenia is a rare condition that can occur as a transient, self-limiting autoimmunity in young children [24] or in a chronic form in adults [25]. The autoantibodies tend to target the FcγRIIIb (CD16),

CD177 or CD11/18 molecules, but can also be HNA specific, for example antibodies with 'relative' HNA-1a specificity are found in as many as 23% of childhood cases of autoimmune neutropenia.

The most sensitive method for the detection of autoantibodies is to test the patient's neutrophils using the direct immunofluorescence test. However, the combination of severe neutropenia, high blood sample volume requirements to recover sufficient neutrophils and the need for a fresh sample limits the applicability of this test, especially in children. Screening of a patient's serum with a panel of typed neutrophils in the indirect granulocyte immunofluorescence or granulocyte agglutination tests provides a suitable alternative, and in some studies this approach has been found to be only slightly less sensitive than performing a direct test.

Persistent Post–Bone Marrow Transplant Neutropenia

Antibody-mediated neutropenia may be a serious complication of bone marrow transplantation [26]. In this context, as the neutrophil antibodies may be autoimmune and/or alloimmune in nature, laboratory investigation requires serological and typing studies to elucidate the nature of the antibodies involved.

Drug-Induced Neutropenia

Drug-induced immune neutropenia (DIIN) occurs when drug-dependent antibodies form against neutrophil membrane glycoproteins and cause neutrophil destruction. Affected patients have fever, chills and infections; left untreated, severe infections can result in death. Severe neutropenia or agranulocytosis associated with exposure to non-chemotherapy drugs ranges from approximately 1.6 to 15.4 cases per million population per year. Unlike for drug-induced immune thrombocytopenia and anaemia, studies of DIIN are limited, so knowledge of possible mechanisms and utility of laboratory testing for antibodies is not well understood [27]. However, in cases of severe neutropenia in which other causes have been ruled out, discontinuation of the suspected drug(s) should be considered.

KEY POINTS

1) Allo-, auto-, iso- and drug-dependent antigens may be found on platelets and neutrophils and are implicated in a range of immune cytopenias.
2) Alloantigens on platelets are known as HPAs; alloantigens on neutrophils are known as HNAs.
3) Reliable detection and identification of HPA- and HNA-specific antibodies requires the use of both whole-cell type assays such as the platelet immunofluorescence test (PIFT) and granulocyte immunofluorescence test by flow cytometry (GIFT-FC), and antigen-capture type assays such as the monoclonal antibody immobilisation of platelet antigens (MAIPA) and monoclonal antibody immobilisation of granulocyte antigens (MAIGA) assays, respectively.
4) HPA and HNA types can mostly be determined using polymerase chain reaction (PCR)-based methodologies.
5) Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a common disorder and HPA-1a or HPA-5b antibodies are responsible for approximately 95% of cases.
6) Optimal postnatal treatment of infants with FNAIT is the transfusion of donor platelets lacking the HPA targeted by maternal antibodies.
7) Optimal antenatal treatment of FNAIT is intravenous immunoglobulin (IvIgG).

8) HNA antibodies can be associated with both alloimmune and autoimmune neutropenia.
9) Transfusion-related acute lung injury (TRALI) can be a life-threatening condition, especially if HNA-3a antibodies are involved.
10) The incidence of antibody-mediated TRALI has been significantly reduced by implementation of a number of different strategies to reduce transfusion of leucocyte antibodies.

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6 Pretransfusion Testing and the Selection of Red Cell Products for Transfusion

Mark H. Yazer[1], Meghan Delaney[2] and Gregory A. Denomme[3]

[1] *Department of Pathology, University of Pittsburgh, Pittsburgh, PA; Centralized Transfusion Service, Pittsburgh, PA, USA; Department of Pathology, Tel Aviv University, Tel Aviv, Israel; Department of Clinical Immunology , University of Southern Denmark, Odense, Denmark*
[2] *Department of Pathology & Laboratory Medicine Division, Children's National Hospital; Professor, Department of Pathology & Pediatrics, The George Washington University, Washington, DC, USA*
[3] *Versiti Blood Research Institute and Diagnostic Laboratories, Milwaukee, WI, USA*

The goal of pretransfusion compatibility testing is to ensure that serologically safe blood products are issued to the recipient. It is therefore crucial to accurately determine the recipient's blood type (ABO group, D type) and whether they have any unexpected red cell antibodies. To this end, a 'type and screen' are often ordered together, although as detailed below they are actually two separate tests.

Determining the Recipient's ABO Group and Screening for Unexpected Antibodies

Clerical Check and Verification of Recipient's Identity

The first step in ensuring the recipient's serological safety that the blood bank performs is a check of the recipient's name and unique identifiers that are supplied on the tube of blood and the requisition that specifies the nature of the testing to be performed. The name and unique identifiers on the tube of blood and the requisition must match exactly, as it has been demonstrated that even a seemingly innocuous discrepancy can lead to an unacceptably high risk for a wrong blood in tube (WBIT) error [1]. WBIT errors, or miscollections, occur when the blood in the tube does not come from the recipient whose name is on the tube. These are serious errors that can lead to ABO-incompatible mistransfusions that can cause morbidity and possibly the death of the recipient. Any samples in which an identification discrepancy has been identified must be rejected, and a new, properly labelled sample obtained for testing. In some countries, it is mandatory for a recipient's ABO group to be tested twice on the same sample if the recipient does not have an ABO group on record at that hospital. This process merely validates the precision of the typing – it does not help to prevent WBIT errors.

To improve the accuracy of the typing, many hospitals have a requirement for a recipient without a historical type to have, at a minimum, their ABO group performed on two separately collected samples before ABO-identical components are issued [2]. If the ABO groups of both samples match each other, confidence that the intended recipient's blood was actually drawn increases.

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Thus, the historical type that is maintained on file functions as an important means of detecting miscollections, and the more recipients who are covered by a database of historical ABO groups, the further their serological safety is enhanced [3–5].

Determining the Recipient's ABO Group

To determine the recipient's ABO group, two complementary tests are used. The forward type (also known as 'front type' or cell typing) is used to detect the antigens on the recipient's red cells using monoclonal immunoglobulin (Ig)M anti-A and anti-B reagent blends and observing for agglutination. These pentameric IgM antibodies can cross-link antigens on adjacent cells, causing direct agglutination of red cells without requiring additional reagents. The reverse type (also known as 'back type' or plasma typing) makes use of the fact that virtually everyone older than a few months of age will have naturally occurring IgM antibodies to the A or B (or both) antigens lacking on their own red cells. These naturally occurring antibodies are called isohaemagglutinins. This test is performed by separately mixing the recipient's serum or plasma with commercially available A_1 and B red cells and observing for agglutination. A reverse type is not performed on neonates, whose serum is not typically tested until they are older than 4 months of age, as any anti-A or anti-B detected in their serum is presumed to be of maternal origin. Expected patterns of agglutination are demonstrated in Table 6.1.

Occasionally, discrepancies between the forward and reverse typings occur. Some common causes of ABO discrepancies include being immunosuppressed, receipt of intravenous immunoglobulin (IvIg) and converting to the donor blood group after a stem cell transplant. Furthermore, genetic subtypes of A and B can also cause weak or absent agglutination on the forward type that can lead to discrepant results [6,7]. In very unusual cases, naturally occurring chimeras or individuals with mosaic phenotypes can produce very confusing and apparently discrepant results [8]. In all situations when an ABO discrepancy is detected, a thorough investigation into its cause should be performed, starting by ensuring that a WBIT error or accidental mistyping in the blood bank did not occur, before group-specific blood products can be issued.

Determining the Recipient's D Type

To determine the recipient's D type, a procedure similar to the forward type is performed. The recipient's red cells are exposed to an IgM monoclonal anti-D reagent that does not detect the DVI variant (because individuals with this phenotype can become alloimmunised if transfused with D-positive red cells; thus as recipients they should be typed as D negative and transfused with D-negative red cells). As with the forward type, agglutination is the positive endpoint of this test. D typing can be complicated when the recipient has weak or partial D alleles [9].

Table 6.1 Expected ABO grouping patterns.

	Forward type		Reverse type	
Group	**Anti-A**	**Anti-B**	**A1 cells**	**B cells**
A	+++	–	–	+++
B	–	+++	+++	–
O	–	–	+++	+++
AB	+++	+++	–	–

Difficulties in D Typing: Weak and Partial D

A weak D allele is one that typically encodes a protein with mutations in its intramembrane or intracellular regions, such that it is unstable in the red cell membrane and fewer than normal numbers of the protein are expressed on the red cell surface (Figure 6.1). The RhD protein is thought to be fully intact with all its epitopes present; there are simply fewer proteins on the surface, resulting in weaker than normal agglutination with some D typing reagents.

Most experts now agree that recipients (and pregnant women) who carry the most common weak D alleles (types 1, 2, 3) should be considered to be D positive, with a low risk of forming anti-D if exposed to D-positive red blood cells (RBCs) from transfusion or pregnancy [10,11]. Performing *RHD* allele genotyping can be helpful in determining a recipient's potential for becoming alloimmunised, although caution is advised if a female of childbearing age with a weak or unusual D phenotype requires an urgent transfusion before the results of her *RHD* genotype are known; in this case D-negative cellular blood products should be used [12].

Partial D recipients, on the other hand, usually type very strongly with anti-D reagents and are often indistinguishable from those with normal D proteins because they have a normal copy number of RhD proteins embedded in their red cell membrane (see Figure 6.1). However, partial D proteins are lacking at least one D epitope, often due to genetic crossover events with the structurally similar *RHCE* gene.

- When partial D recipients are exposed to D-positive red cells, they can become D alloimmunised.

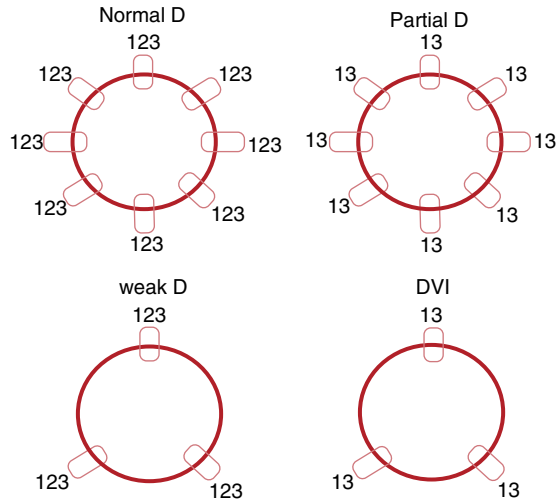

Figure 6.1 Comparison of weak D and partial D with a normal D-positive red blood cell (RBC). The circles represent the RBC membrane, the rectangles represent an RhD protein and the numbers above each RhD protein are a stylised representation of different D epitopes on the protein. The D epitopes are arbitrarily numbered 1, 2 and 3. The number of antigens and epitopes, as well as the size of the RhD protein, is not to scale. In this example, eight D antigens on the RBC surface are schematically shown as normal, and each D antigen has three D epitopes. In reality, the number of D antigens ranges from 10 000 to 25 000 and more than 30 D epitopes are expressed on the D antigen. The weak D RBC features D antigens with the full complement of D epitopes, but the number of D antigens is reduced compared to normal. The partial D RBC demonstrates the normal number of D epitopes, but each protein is lacking at least one D epitope. The partial D type DVI demonstrates both weak D and partial D features. *Source:* This figure originally appeared in Flegel WA, Denomme GA, Yazer MH. On the complexity of D antigen typing: a handy decision tree in the age of molecular blood group diagnostics. J Obstet Gynaecol Can 2007;**29**:746–52, and is reprinted here with the kind permission of Elsevier.

- Detecting partial D recipients before they are mistaken for normal D-positive recipients can usually only be done using *RHD* genotyping methods.
- The usual presentation of a partial D recipient in the blood bank is the conundrum they create when they present following transfusion of D-positive red cells with an allo anti-D.

Both weak and partial D recipients are uncommon. The Further Reading list at the end of the chapter provides some additional sources for more detailed information on the genetics, frequency and management of donors and recipients with these alleles.

Antibody Screening and Identification

Overall, fewer than 5% of transfused recipients will develop an antibody to foreign red cell antigens other than A or B. This percentage is often much higher in sickle cell disease patients, where the alloimmunisation rate can approach 50% [5,13]. Hence, these antibodies are collectively referred to as 'unexpected antibodies' due to their relative rarity. However, it is important to detect red cell antibodies when they are present, because transfusing a recipient who has an unexpected antibody with antigen-positive red cells can result in a variety of outcomes, ranging from shortening the lifespan of the transfused red cell without significant untoward consequences for the recipient to an outright immediate haemolytic reaction with severe clinical consequences. Although most red cell antibodies are allogeneic (following exposure to foreign red cell antigens via transfusion or pregnancy), some are autoantibodies that do not require exposure to foreign red cells for development.

To detect the presence of unexpected red cell antibodies, the recipient's serum/plasma should be tested against two or more reagent screening RBCs (hence the meaning of the second part of the phrase 'type and screen'). The reagent screening cells are always group O and should between them express all the clinically significant antigens; ideally the Rh phenotypes R_1R_1, rr and R_2R_2 should be represented in the screening cell set. Different national standards and guidelines exist, but in many countries it is recommended that the screening cells express the Jk^a, Jk^b, S, s, Fy^a and Fy^b antigens, and incorporate the following phenotypes: Jk(a+b–), Jk(a–b+), S+s–, S–s+, Fy(a+b–), Fy(a–b+), since stronger reactions may be obtained with cells expressing double-dose antigen expression.

If an unexpected red cell antibody is detected in the antibody detection (screening) test, the blood bank must identify its specificity and, when clinically significant, select antigen-negative units for crossmatching (see below). The antibody's specificity is determined by testing the recipient's serum/plasma against a large panel of reagent group O red cells of known phenotypes (i.e. the antigens on the surface of the red cells). The reagent red cells in the panel used to determine the specificity of an antibody are similar to those used in the screen, but because typically 10–11 reagent red cells are used in the panel (compared to only 2–3 in the screen), the antibody's specificity can be determined. Antibody specificity can be assigned when the serum/plasma is reactive with at least two examples of red cells bearing the antigen, and non-reactive with at least two examples of red cells lacking the antigen.

Antibody Detection Methods

Test methods have been developed to detect antibodies of different isotypes. Antibodies with specificities for red cell antigens are usually IgG or IgM. As stated above, pentameric IgM antibodies can cross-link antigens on adjacent cells, causing direct agglutination of red cells. Conversely, IgG antibodies are monomeric and, although divalent, the distance between the Fab regions on a single IgG molecule is generally insufficient to allow for direct agglutination. That is, the antigen density on the red cells is usually insufficient to permit the Fab regions of IgG molecules to span the distance between two adjacent red cells and cause direct agglutination. Methods such as the antiglobulin test

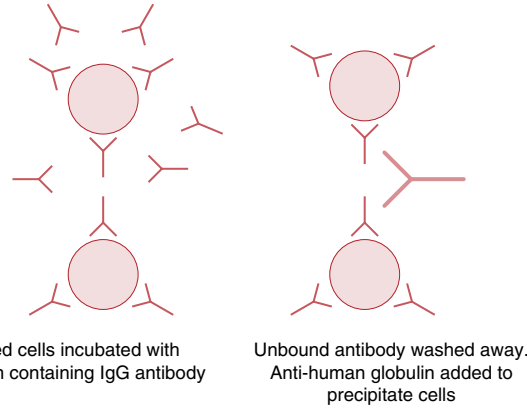

Figure 6.2 Indirect immunoglobulin test.

(sometimes referred to as the Coombs' test or antihuman globulin test) that use a secondary anti-isotype antibody (Figure 6.2) or the enzyme method (which uses proteolytic enzymes such as papain or ficin to cleave negatively charged, hydrophilic residues from red cell membranes) must therefore be used to detect most IgG red cell antibodies.

Test systems for detection of serological reactions can be classified into three broad categories.

Liquid-Phase ('Tube') Systems

Liquid-phase systems rely on visualisation of haemagglutination reactions in individual glass/plastic test tubes or microplates. The presence or absence of agglutinated red cells distinguishes positive and negative reactions, allowing grading of reaction avidity according to strength of haemagglutination. While not the most sensitive methods available today, liquid-phase testing methods using red cells suspended in low ionic strength solution (LISS) remain the gold standard for the detection of clinically significant red cell alloantibodies. Using polyethylene glycol (PEG) is another enhancement technique.

Column-Agglutination Systems

Introduction of column-agglutination systems (commonly referred to as 'gel') has resulted in significant changes to routine laboratory practice. Synthetic gel mixtures or glass microbeads configured into vertical columns on small cards form density barriers, retaining agglutinated red cells and allowing passage of the non-agglutinated cells. Positive reactions (antibody/antigen interactions) are distinguished by agglutinates at or near the top of the gel column and negative reactions appear as buttons of red cells at the bottom (Figure 6.3).

Reagent (IgM) antibody can be incorporated into the columns, allowing phenotyping simply by addition of test cells to the top of the column. Similarly, the implicit association test (IAT) can be performed in columns containing antiglobulin reagent to which plasma and reagent red cells are added. Manual and automated methods for performing and interpreting these tests are now widely available.

Solid-Phase Systems

These systems use microplates for testing. The positive reaction endpoint is characterised by a red cell monolayer in the wells, while discrete buttons of red cells at the bottom of the well indicate negative reactions (Figure 6.4). Generally, solid-phase tests are performed using automated instruments that provide an interpretation of the result.

Autoantibodies

Autoantibodies may be suspected when the recipient's serum/plasma reacts with the A_1 and B cells used in the reverse ABO group

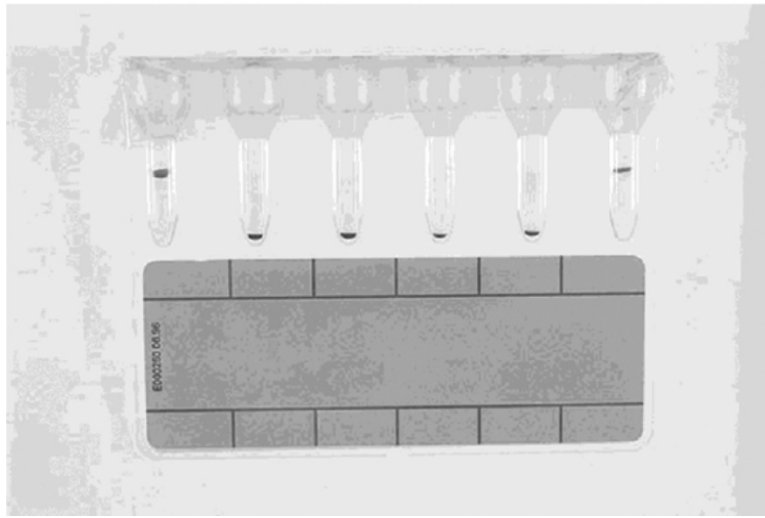

Figure 6.3 Column-agglutination technology for blood grouping and antibody screening. Samples may consist of patient cells and reagent antisera or reagent red cells and patient serum/plasma. Positive results are seen in the first and last columns; the other columns show negative reactions.

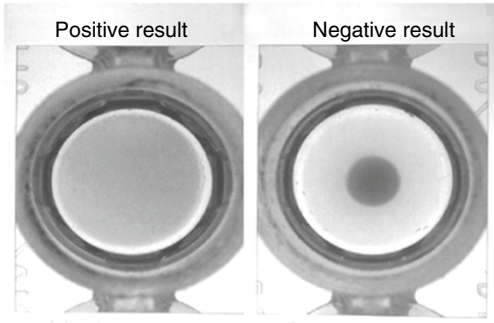

Figure 6.4 Solid-phase blood grouping technology.

(cold autoantibodies) or with all cells in the antibody screen and antibody identification panel, including the patient's own red cells. Autoantibodies are found in a minority of patients, but because they lead to interference in blood typing and antibody screening, the laboratory evaluation of autoantibodies is complex. Not all autoantibodies give rise to clinically significant haemolysis. Serological investigations should focus on determining the patient's ABO and D group and excluding the presence of underlying alloantibodies. Cold-type autoantibodies tend not to confound alloantibody identification unless they react at 37 °C, although they can interfere with the reverse typing test. Warm-type autoantibodies will usually cause the patient's red cells to have a positive direct antiglobulin test (DAT) due to coating with IgG with or without complement, and an eluate prepared from these cells typically reacts with all the panel cells. Chapter 32 has more information on the clinical management of patients with immune-mediated haemolysis. To avoid the interference posed by warm-type autoantibodies, laboratories use techniques to remove or adsorb out the autoantibody.

Pretransfusion Molecular Testing

Pretransfusion molecular testing, also known as red cell genotyping, using genomic DNA as the biological source rather than red cells, has gained clinical usefulness in an effort to avoid red cell alloimmunisation and the potential for immune-mediated adverse reactions associated with transfusion. On the one hand, ABO and RhD-matched blood is standard practice for the provision of blood, but we now know that pretransfusion antigen-matched blood provides the chronic transfusion recipient with another level of

safety. Approximately 30% of recipients who are chronically transfused with a red cell product will make an alloantibody. It makes sense to consider antigen matching in specific instances to avoid alloimmunisation. Pretransfusion phenotyping can be used to determine the antigen status of a transfusion recipient. Genotyping also can be performed using licensed reagents/platform. It is accurate, provides the same information as a phenotype in an efficient manner, and can be done regardless of recent transfusion or when red cells are coated with IgG.

There are three clinical settings wherein chronic transfusion recipients benefit from the provision of antigen-matched blood: haemoglobinopathies, warm autoimmune haemolytic anaemia and anti-CD38 therapy. Haemoglobinopathies require special consideration. The worldwide standard of care is to provide RhD-, C-, E-, c-, e- and K-matched blood to avoid alloimmunisation to these blood group antigens [14]. Recipients are matched for the principal antigens of the MNS, K, Fy and Jk antigens once they are alloimmunised to any blood group antigen. Because many of these recipients harbour unusual RH, MNS, or Duffy variants, genotyping platforms include the identification of the alleles linked to variants within these three blood group systems. It is not possible or practical to match for these variants. However, knowing that such variants are present can help alert the laboratory to the potential for alloimmunisation due to the expression of a partial antigen or the lack of a high-prevalence antigen. This information makes antibody identification somewhat easier. Genotyping for the Fy blood group system has important application. The presence of the promoter mutation leads to the erythrocyte suppression of the Fy^b antigen. These transfusion recipients are tolerant of Fy^b and can safely be transfused with Fy(b+) blood [15]. This practice allows for more blood to be a potential match, given that some 40–50% of patients diagnosed with a haemoglobinopathy are Fy(b–) due to the promoter mutation. Antigen matching in the setting of warm autoimmune haemolytic anaemia and anti-CD38 therapy provides a safe alternative to repetitive and time-consuming antibody investigation workups [16,17]. These transfusion recipients can receive MNS, RH, K, Fy, and Jk antigen-matched blood, with minimal effort beyond electronic confirmation of the ABO/RhD when the crossmatch is incompatible.

Crossmatching Techniques

The crossmatch ensures that an ABO-compatible red cell has been selected for transfusion, and that the unit is antigen negative in the case of a recipient with an unexpected antibody.

Crossmatch Techniques for Patients without Current or Historical Antibodies

If a recipient has a negative antibody screen and no record of historical red cell antibodies, an immediate spin (IS) crossmatch can be used to issue red cells. The recipient's plasma is mixed and centrifuged with the potential donor's red cells. As anti-A and anti-B are IgM antibodies that can directly agglutinate red cells, this IS crossmatch provides a final confirmation of ABO compatibility between the recipient and the blood donor. There is negligible risk in omitting the IAT crossmatch in recipients with a negative screen [18]. Antibodies directed against low-frequency antigens may be missed, but the majority of these are clinically insignificant, meaning that they do not cause haemolysis. False-positive IS results arising from rouleaux or cold agglutinins can occur and can also cause ABO discrepancies.

Many centres now perform electronic crossmatching (also known as the computer crossmatch) to confirm donor/recipient ABO compatibility before release of ABO type-specific RBCs as a surrogate for the IS crossmatch. There are several essential prerequisites for using this technique:

- The computer system contains logic to prevent the assignment and release of ABO-incompatible blood, including 'hard

stops' to prevent the release of ABO-incompatible blood.
- No clinically significant antibodies are detected in the recipient's current antibody screen and there is no record of the transfusing facility having previously detected such antibodies.
- There are concordant results of at least two determinations of the recipient's ABO and D groups on file, at least one of which is from a current sample.
- Critical system elements (application software, readers and interfaces) have been validated on site, and there are mechanisms to verify the correct entry of data prior to the release of red cell units, such as barcode identifiers to enter information when it cannot be automatically transferred.

Electronic red cell issue has been widely used for over a decade and is now routine practice in many countries. It has several advantages over serological crossmatching:

- Reduced technical workload.
- Rapid availability of blood. The electronic crossmatch can be performed in under 5 minutes, whereas even the fastest serological crossmatch technique requires at least 20 minutes.
- Improved blood stock management through reduced numbers of crossmatched red cells and reduced wastage.
- Less handling of biohazardous material.
- Elimination of insignificant false-positive results in the IS.
- Ability to issue blood electronically at remote sites, using trained non-laboratory staff.

This last characteristic has allowed the development of systems for electronic remote blood issue. When patient details are entered, the system checks that criteria for electronic issue are fulfilled, and either allows access to ABO- and D-compatible units in the remote blood refrigerator or dispenses compatible units. A compatibility label is printed and attached to the unit and rescanned to ensure it is the correct one for the unit. Such systems reduce the time taken for the issue of blood, particularly in small hospitals without transfusion laboratories [19].

Crossmatch Techniques for Patients with Current or Historical Antibodies

For patients with current or historical antibodies, a full IAT crossmatch is required. This means that the recipient's plasma is mixed with the potential donor's red cells, and the antihuman globulin reagent is added. The absence of agglutination or haemolysis indicates compatibility between recipient and donor. This type of crossmatch takes approximately 45 minutes to perform, and thus extra time is required to provide red cells to recipients with antibodies.

Selection of Red Cells for Transfusion

Selecting ABO-Compatible Red Cells

Table 6.2 shows the donor ABO groups that are compatible with the recipient's ABO group. For patients without antibodies, ABO compatibility is the only necessary compatibility consideration.

Selecting D-Compatible Red Cells

Typically, D-negative recipients should receive D-negative RBCs. However, in circumstances when the D-negative RBC inventory is unusually low or in an emergency situation when the recipient's D status is unknown, some transfusion services will issue D-positive RBCs to selected D-negative recipients who meet certain criteria. These criteria usually consider the age and sex of the recipient, as well as whether the recipient has a history of having formed anti-D. These switching strategies are designed to preserve the D-negative RBC inventory for D-negative females of childbearing age and children. Sometimes a lower age threshold for switching a D-negative male compared to a D-negative female to D-positive RBCs is employed, as alloimmunisation in the former does not carry the risk of causing haemolytic disease

of the fetus and newborn (HDFN). The rate of D alloimmunisation of hospitalised D-negative recipients of at least one unit of D-positive RBCs has been shown to be approximately 22%. The benefits of providing life-saving transfusions to massively bleeding patients should outweigh the small risk of D alloimmunisation; one study estimated the risk of severe HDFN following the transfusion of a massively bleeding D-negative female of childbearing age with D-positive RBCs at 0.3% [20].

Clinical Significance of Unexpected Antibodies

Table 6.3 lists some of the more common unexpected antibodies and suggests how to select and crossmatch red cells for recipients with these antibodies. Most clinically significant antibodies are of the IgG isotype.

Table 6.2 ABO compatibilities between donor and recipient for red cell and plasma-containing products.

Donor ABO group	Compatible red cell donor	Compatible plasma-containing product donor
A	A, O	A, AB
B	B, O	B, AB
O	O only	All ABO groups
AB	All ABO groups	AB only

Table 6.3 Recommendations for selection of blood for patients with red cell alloantibodies.

	Typical examples	Procedure
Antibodies considered clinically significant	Anti-RhD, -C, -c, -E, -e Anti-K, -k Anti-Jk^a, -Jk^b Anti-S, -s, -U Anti-Fy^a, -Fy^b	Select ABO-compatible, antigen-negative blood for serological crossmatching
Antibodies directed against antigens with an incidence of < 5%, and where the antibody is often not clinically significant	Anti-C^w Anti-Kp^a Anti-Lu^a Anti-Wr^a (anti-Di3)	Select ABO-compatible blood for serological crossmatching
Antibodies primarily reactive below 37 °C, and never or only very rarely clinically significant	Anti-A_1 Anti-N Anti-P_1 Anti-Le^a, -Le^b, -Le^{a+b} Anti-HI (in A_1 and A_1B patients)	Select ABO-compatible blood for serological crossmatching, performed strictly at 37 °C
Antibodies sometimes reactive at 37 °C and clinically significant	Anti-M	If reactive at 37 °C, select ABO-compatible, antigen-negative blood for serological crossmatching If unreactive at 37 °C, select ABO-compatible blood for serological crossmatching, performed strictly at 37 °C
Other antibodies active by IAT at 37 °C	Many specificities	Seek advice from blood centre

IAT, implicit association test.

Use of Uncrossmatched Red Cells

As group O red cells are the 'universal donor' type, they can be safely administered to recipients of any ABO blood group. Group O red cells may be issued in life-threatening situations where even the short time required to perform pretransfusion testing would jeopardise a bleeding recipient's life. In these emergency situations, if the patient is a premenopausal female, group O-negative uncrossmatched red cells should be used if available, but a life-saving transfusion with D-positive RBCs should not be withheld out of fear of future HDFN. Otherwise, if the patient is an older female or a male, O-positive units can be selected. Group-specific units can be provided as soon as the patient's group is known.

Concerns about the potential for haemolysis to occur when uncrossmatched units are used sometimes arise, because often these units are transfused before the antibody screen is complete. Thus there is a potential for recipients with antibodies to receive uncrossmatched units that bear the antigen to which they have become sensitised. Fortunately, even in these cases when an incompatible uncrossmatched unit has been transfused, the risk of overt haemolysis is quite low.

- In a study of seven recipients of uncrossmatched red cells who received at least one unit that was incompatible with their antibody, only one recipient actually demonstrated biochemical evidence of haemolysis, although it was unclear whether the patient haemolysed the uncrossmatched unit or one of the earlier units that he had received to treat his gastrointestinal bleed (a condition that could also confound the interpretation of the biochemical markers) [21].
- Overall, of the 265 emergency-issued red cells in that study, this was the only reported haemolytic event, representing only 0.4% of all of the uncrossmatched units.
- A low rate of haemolysis following uncrossmatched red cell transfusion was also found in several other studies (Table 6.4).

Table 6.4 Summary of clinical studies on the rate of haemolysis following the transfusion of uncrossmatched red cells. See original text for complete reference citations.

Study	Number of recipients	Number of uncrossmatched red cell units issued	Rate of haemolysis	Rate of new antibody formation
Mulay 2012	1407	4144	1/1407 (0.02%)	7/232* (3%)
Radkay 2012	218	1065	1/218 (0.5%)	4/218 (1.8%)
Miraflor 2011	132	1570	1/132 (0.8%)	1/132
Goodell 2010	262	1002	1/262 (0.4%)	Not reported
Ball 2009	153	511	0	Not reported
Dutton 2005	161	581	0	1/161 (0.6%)
Unkle 1991	135	Not reported	0	3/135 (2.2%)
Lefebre 1987	133	537	0	Not reported
Schwab 1986	99	410	0	Not reported
Gervin 1984	160	875	0	Not reported
Blumberg 1978	46	221	0	Not reported
Total	2906	10 916	4/2906 (0.1%)	16/878 (1.8%)

Source: Boisen et al. [25]. Reproduced with permission of Wolters Kluwer Health Inc.

- Thus, if a patient is exsanguinating and requires urgent red cell transfusion before the type and screen are finished, uncrossmatched red cells can be a safe and life-saving intervention [22].

Selection of Platelets and Plasma Components

ABO- and D-compatible platelets are preferable, but when these are not available, ABO- and D-incompatible platelets may be used in adults. Whether to administer RhIg following the transfusion of D-positive platelets to a D-negative recipient depends on the patient's age, sex, probability of receiving additional transfusions in the future, immune status and so on. The D alloimmunisation rate following D-positive platelet transfusion to a D-negative recipient is much lower than with RBCs at 1.4% [23].

Under routine circumstances, plasma should be ABO compatible with the recipient, and AB plasma is the universal plasma donor group (see Table 6.2). In a bleeding emergency, many centres now use group A plasma even if the recipient's type is unknown, with or without performing a titre on the anti-B [24]. In some countries, plasma can only be maintained in the thawed state for up to 24 hours at refrigerator temperatures (1–6 °C), while in others, the plasma can be kept in the refrigerator for an additional four days after thawing. Thawed plasma has the advantages of being readily available for issue and reducing waste. Other preparations, such as pooled, solvent-detergent plasma or plasma that has been virally inactivated using other techniques, are also available in some regions.

Cryoprecipitate is prepared from whole-blood donations. It should ideally be of the same ABO group as the recipient, but in adults this is not essential due to its very small volume. Cryoprecipitate is mainly used as a source of fibrinogen, where a virally inactivated fibrinogen concentrate is not available.

Frozen products must always be thawed using an approved device and method.

KEY POINTS

1) Pretransfusion testing establishes the recipient's ABO group and D type, and also detects unexpected antibodies. This process can take several hours or longer, depending on the number and nature of the antibodies present, so it is always best ordered far in advance of the patient's surgery or procedure to avoid delays in obtaining crossmatched red cells.
2) Distinguishing between weak and partial D phenotypes is not always possible with serological methods and a genotype should be obtained in patients with unusual or discrepant D phenotypes.
3) Electronic red cell crossmatching is a time-saving technique and can be used in the vast majority of recipients when working in a laboratory with a validated computer system.
4) Uncrossmatched red cells, when used in emergency life-threatening situations, pose a low risk of haemolysis to the recipient and can be life-saving.

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7 Investigation of Acute Transfusion Reactions

Heather Leary[1], *Kathryn E. Webert*[2] *and Nancy M. Heddle*[3]

[1] *Department of Haematology, Churchill Hospital, Oxford, UK*
[2] *Canadian Blood Services, and Department of Medicine and Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada*
[3] *Department of Medicine, McMaster University, and Canadian Blood Services, Hamilton, Ontario, Canada*

The investigation of suspected acute reactions to blood components and plasma derivatives cannot be summarised in a single simple algorithm for several reasons:

- Signs and symptoms are not specific for one type of reaction.
- The frequency and type of reactions vary with different blood components, e.g. leucocyte reduced or not.
- Risks are variable with different patient populations.
- The severity and risk of reactions must be taken into account to ensure a balance between the safety, availability and costs due to wastage.

In this chapter, an algorithmic approach is provided for the clinical management and laboratory investigation of transfusion reactions.

Understanding the Clinical Presentation and Differential Diagnosis

Acute reactions are defined as adverse events occurring during or within 4–6 hours of transfusion. They can usually be placed into the following categories [1,2]:

- Acute haemolysis (AHTR).
- Allergic.
- Anaphylactic.
- Transfusion-related acute lung injury (TRALI).
- Febrile non-haemolytic reactions (FNHTR).
- Bacterial sepsis.
- Hypotension.
- Transfusion-associated circulatory overload (TACO).
- Transfusion-associated dyspnoea (TAD).
- Acute pain reaction.
- Metabolic complications (hyperkalaemia, hypokalaemia, hypocalcaemia, hypothermia).

There are other types of reactions that can occur following the acute period, including delayed haemolytic reactions, transfusion-associated graft-versus-host disease, posttransfusion purpura, alloimmune thrombocytopenia and alloimmune neutropenia. These reactions are discussed in other chapters.

The diagnosis of an acute transfusion reaction can be challenging, as signs and symptoms are not specific for each type of reaction, all possible signs and symptoms do not present with every reaction and different types of reactions can occur simultaneously. In Table 7.1, signs and symptoms have been

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grouped into nine categories. The information summarised in this table illustrates how similar signs and symptoms can occur in different reactions (e.g. bacterial sepsis, allergic and anaphylactic reactions can all present with cutaneous symptoms).

To ensure management strategies and investigations that minimise risks to patients, healthcare professionals need to understand the aetiology and pathophysiology of each type of acute reaction (Table 7.2). It is also essential to understand the typical clinical presentation for each type of reaction so that a differential diagnosis can be formulated as part of the investigative process. Some considerations to assist in the decision-making process and investigation are summarised below [3,4].

Patient History

- The reason for the patient's admission and current diagnosis may give some indication as to the type of reaction. For example, if the patient is being transfused because of anaemia but is also in congestive heart failure, TACO could be the cause of the reaction.
- Consider whether the patient has been previously transfused or pregnant as this can lead to alloimmunisation to red cell and leucocyte antigens, which are known to be associated with certain types of reactions (acute haemolytic transfusion reaction, FNHTR).
- What blood components have been transfused and what is the transfusion timeline? If plasma-containing products have recently been transfused, consider whether the reaction could be caused by passive infusion of antibody or soluble allergens that may now be reacting with the product being transfused.
- Has the patient had a history of reactions when blood components are transfused? Some patients are prone to developing recurrent FNHTR and/or allergic reactions when transfused.
- Is the patient known to be IgA deficient? Some patients with IgA deficiency develop anti-IgA antibodies, which may cause anaphylactic transfusion reactions when an IgA-containing blood component is transfused.

Medications

Determine what medications the patient is receiving or has received in the time period leading up to the transfusion. Considerations should include:

- The use of premedications given to prevent acute reactions such as allergic (antihistamines) or FNHTR (antipyretics).
- Antimicrobial medication.
- Pyrogenic agents that are known to cause fever, such as amphotericin or monoclonal antibodies.
- Angiotensin-converting enzyme (ACE) inhibitors, which have been associated with hypotensive reactions.
- Pruritogenic agents, for example vancomycin, narcotics.

Type of Blood Component Being Transfused

- Does the component contain a significant volume of plasma? Infusion of plasma is associated with a variety of reactions, including allergic, anaphylactic, TRALI and acute haemolysis caused by passive antibody incompatibility with the patient's red cells [3,4].
- Does the component contain a significant number of red cells? If greater than 50 mL of red cells are present in the component, acute haemolysis needs to be considered as a possible cause of the adverse reaction.
- Was the component stored at room temperature or in a refrigerator? Platelets have a higher risk of bacterial contamination if they are stored at room temperature. However, products stored at colder temperatures can also be contaminated with bacteria, especially those strains that are known to grow at cold temperatures [3].
- Is the component leucocyte reduced and, if so, was leucocyte reduction performed

Table 7.1 Summary of the signs/symptoms typically observed with different types of acute transfusion reaction.

Reaction type	Cutaneous	Pain	Inflammatory	Respiratory	Hypotension	Hypertension	Other cardiovascular	GI	Neuromuscular	CNS	DIC	Haemoglobinuria	Renal failure
AHTR		√		√	√						√	√	√
Allergic	√			√									
Anaphylactic	√		√	√	√	√	√	√					
TRALI			√	√	√		√						
FNHTR		√	√					√					
Bacterial sepsis	√	√	√	√	√	√	√	√		√	√	√	
Hypotensive		√	√	√	√								
TACO		√		√		√	√	√					
Acute pain		√	√	√		√	√		√				
Hyperkalaemia							√	√	√				
Hypokalaemia		√					√		√	√			
Hypocalcaemia		√	√				√	√	√				
Hypothermia				√			√						
Hypotensive		√	√	√	√								
TAD				√									

*Flushing only.

AHTR, acute haemolytic transfusion reaction; CNS, central nervous system; DIC, disseminated intravascular coagulation; FNHTR, febrile non-haemolytic transfusion reaction; GI, gastrointestinal; TAD, transfusion-associated dyspnoea; TACO, transfusion-associated circulatory overload; TRALI, transfusion-related acute lung injury.

Table 7.2 Summary of acute transfusion reactions. *Source*: Data from Callum et al. [3] and Popovsky [4].

Reaction	Frequency	Mechanism	Clinical presentation	Differential diagnosis	Laboratory investigations	Management
AHTR	Approximately 1 : 30 000 red cell and platelet transfusions Also with IvIg Fatal reaction 1 : 600 000	Results from the destruction of donor red cells by preformed recipient antibodies	Fever, flank pain and red/brown urine	FNHTR, bacterial contamination, TRALI, Non-immune causes of haemolysis	Positive DAT-free haemoglobin in plasma and urine Positive crossmatch	Stop the transfusion immediately. Begin infusion of normal saline
		Antibodies fix complement and cause rapid intravascular haemolysis	Hypotension, shock, death			Alert the blood transfusion laboratory, check for clerical error, send entire transfusion set-up to blood transfusion laboratory for testing
		Usually due to ABO incompatibility, which is most often the result of clerical error				Obtain blood samples: DAT, plasma for free haemoglobin, antibody screen Obtain urine sample: haemoglobinuria
		Acute haemolysis can be seen with IvIg due to the presence of anti-A and anti-B antibodies in the product				

Allergic transfusion reaction	More common in platelet transfusions, 1 : 2000, and plasma transfusions, 1 : 8000, than red cell transfusions, 1 : 30 000	Soluble allergenic substances in the plasma of the donated blood product react with preexisting IgE antibodies in the recipient	Hives, urticaria, flushing	Anaphylactic transfusion reaction TRALI TACO	Rule out anaphylactic reaction	Stop the transfusion until a more serious reaction is ruled out Antihistamine may improve symptoms
		Causes mast cells and basophils to release histamine, leading to hives or urticaria				If no evidence of dyspnoea or anaphylaxis, the transfusion may be continued with close observation
Anaphylactic transfusion reaction	1 : 25 000 plasma transfusions	Usually due to the presence of anti-IgA antibodies in recipients who are IgA deficient	Rapid onset of shock, hypotension, angioedema and respiratory distress (2° to bronchospasm and laryngeal oedema)	Allergic transfusion reaction, TRALI, TACO	IgA level, testing for anti-IgA (if IgA deficient)	Stop the transfusion, adrenaline, airway maintenance, oxygenation. Maintain haemodynamic status (IV fluids, vasopressor medications)
TRALI	Confirmed TRALI 1 : 250 000 and possible TRALI 1 : 80 000 red cell, platelet and plasma transfusions	Antibodies or neutrophil-priming agents in the infused blood product likely to interact with the recipient's leucocyte antigens	Shortness of breath, fever, hypotension or hypertension acute noncardiogenic pulmonary oedema (elevated JVP, bilateral lung crackles)	Bacterial contamination, TACO, TAD, anaphylactic transfusion reaction, cardiogenic pulmonary oedema, ARDS pneumonia	Antigranulocyte or anti-HLA antibodies in the donor, CXR (bilateral pulmonary infiltrates), BNP (possibly useful)	Stop the transfusion Respirator support as required (supplemental oxygen, mechanical ventilation). Maintain haemodynamic status (IV fluids, vasopressor medications)

(*Continued*)

Table 7.2 (Continued)

Reaction	Frequency	Mechanism	Clinical presentation	Differential diagnosis	Laboratory investigations	Management
TRALI (*cont'd*)		Activation of the WBC results in the production of inflammatory mediators that increase vascular permeability Leads to capillary leak and pulmonary tissue damage				
FNHTR	Most common in red cell transfusions 1 : 800 and platelet transfusions 1 : 650, and less common with plasma transfusions 1 : 10 000	Likely caused by cytokines that are generated and accumulate during the storage of blood components Less frequently caused by leucocyte antigen/antibody interactions between recipient and blood product	Fever, rigors, chills Other: nausea, vomiting, dyspnoea, hypotension Typically occurs during or within 2 hours of transfusion, but may present up to 6 hours after transfusion	AHTR, Bacterial contamination, TRALI, co-morbid conditions causing fever (i.e. infection, haematological malignancies, solid tumour)	No specific tests Rule out other transfusion reactions	Stop the transfusion until a more serious reaction is ruled out Antipyretics to decrease fever and meperidine may help patients with severe chills and rigors
Bacterial contamination	More common in platelet transfusion 1 : 20 000 and less common in red cell and plasma transfusion 1 : 150 000	Bacteria in the blood product from donor skin (venepuncture site); donor with bacteraemia; contamination during collection /storage	High fever, rigors, hypotension	Drugs causing fever, AHTR, FNHTR, allergic transfusion reaction	Gram stain and culture of remaining blood component Gram stain and culture of patient's blood	Stop the transfusion IV fluids, broad-spectrum antibiotics
Hypotensive transfusion reaction	Most common in red cell and platelet transfusions 1 : 25 000	Unknown		AHTR, bacterial contamination, TRALI, anaphylactic transfusion reaction		Stop the transfusion
		May be related to generation of bradykinin and/or its active metabolite	Hypotension Dyspnoea, urticaria, flushing, pruritus, GI symptoms		No specific tests Rule out other transfusion reactions	Maintain haemodynamic status (IV fluids, vasopressor medications)

	Some reactions may occur during transfusion of blood components administered through a negatively charged filter or to patients receiving an ACE inhibitor	Most reactions occur within minutes of the beginning of the transfusion and resolve rapidly with cessation of the transfusion and supportive care	Unrelated to blood transfusion (i.e. due to blood loss)			
TACO	More common in red cell transfusion 1 : 4000 and platelet transfusion 1 : 8000 than plasma transfusion 1 : 25 000	Increase in central venous pressure, increase in pulmonary blood volume and decrease in pulmonary compliance, with resultant secondary congestive heart failure and pulmonary oedema	Elevated JVP, Bilateral crackles on auscultation, hypertension dry cough, orthopnoea, pedal oedema	TRALI, anaphylactic transfusion reaction, TAD	Chest X-ray Clinical examination BNP (possibly useful)	Stop the transfusion Supplemental oxygen Diuretics
Acute pain reaction	Approximately 1 : 15 000 transfusions	Unknown	Acute pain during transfusion (chest, abdominal, back or flank) Other symptoms include dyspnoea, hypertension, chills, tachycardia, restlessness, flushing, headache	AHTR TRALI TACO Allergic	No specific tests	Temporarily stop transfusion Rule out other causes of reaction Pain management

(*Continued*)

Table 7.2 (Continued)

Reaction	Frequency	Mechanism	Clinical presentation	Differential diagnosis	Laboratory investigations	Management
Hyperkalaemia	Unknown Likely more common in infants and children and individuals receiving massive transfusion	During storage of red blood cells, increasing potassium concentration of the supernatant occurs	Muscle weakness Cardiac effects: ECG changes (e.g. peaked T waves, loss of P wave amplitude, prolonged PR interval and QRS duration), arrhythmias, cardiac arrest, death		Electrolytes ECG	May possibly be prevented by such modalities as slow rate of infusion, use of fresher blood, washing of red cells, use of inline potassium filters (in development)
Hypokalaemia	Rare May occur in association with massive transfusion (especially large amounts of FFP)	Unknown Possibly caused by metabolic alkalosis secondary to citrate metabolism, release of catecholamines, aldosterone and/or antidiuretic hormone	ECG changes (flattened or inverted T waves, U wave, ST depression, wide PR interval), muscle weakness, cardiac arrythmias	Rapid infusion of other solutions low in potassium	Electrolytes, ECG	Consider replacement of potassium
Hypocalcaemia	Rare, but may occur in association with massive transfusion	May occur in massive transfusion recipients with liver failure. Liver normally rapidly metabolises transfused citrate; if rate of citrate delivery exceeds liver's ability to clear citrate, citrate may be able to bind to calcium, resulting in hypocalcaemia	ECG changes (prolongation of QT interval), depressed left ventricular function, increased neuromuscular excitability, hypotension		Calcium level Magnesium level (also bound by citrate)	Consider calcium replacement when ionised calcium concentration is less than 50% of normal value with symptoms of hypocalcaemia

Hypothermia	Unknown Most commonly seen with rapid massive transfusion of red cells (stored between 1 ° and 6 °C)	Occurs with rapid transfusion of large volumes of cold blood (red cells)	Decreased core body temperature Hypothermia may be associated with metabolic derangements (hyperkalaemia, increased lactate, increased oxygen affinity of haemoglobin), abnormalities in haemostasis and cardiac disturbances	Other causes associated with massive transfusion and trauma include infusion of cold fluids, opening of body cavities due to injuries, impaired thermoregulatory control	Not Applicable	May be prevented by use of blood warmers when rapid massive transfusion required
Transfusion-associated dyspnoea	Variable reports, but more common in platelet transfusions, 1 : 20 000 than red cell or plasma transfusions, 1 : 100 000	Unknown	Respiratory distress that does fulfil criteria for TACO or TRALI and cannot be adequately explained by patient's underlying condition	TACO TRALI Anaphylactic transfusion reaction	Not Applicable	Diagnosis of exclusion if history does not fulfil criteria for TACO or TRALI

ACE, angiotensin-converting enzyme; AHTR, acute haemolytic transfusion reaction; ARDS, adult respiratory distress syndrome; BNP, brain natriuretic peptide; CXR, chest x-ray; DAT, direct antiglobulin test; ECG, electrocardiogram; FFP, fresh frozen plasma; FNHTR, febrile non-haemolytic transfusion reaction; GI, gastrointestinal; HLA, human leucocyte antigen; Ig, immunoglobulin; IV, intravenous; IvIg, intravenous immunoglobulin; JVP, jugular venous pressure; TACO, transfusion-associated circulatory overload; TAD, transfusion-associated dyspnoea; TRALI, transfusion-related acute lung injury; WBC, white blood cell.

Source: Quantitative data from Heddle N et al., Ontario Transfusion Transmitted Injuries Surveillance System (TTISS) Program Report: 2014–2018, https://ttiss.mcmaster.ca/wp-content/uploads/2019/12/Final-OntarioTTISS-Report-5-Year-2014-2018-Dec-6-2019.pdf

before or after storage? Non-leucocyte-reduced blood components (especially platelets) are associated with a higher frequency of FNHTR. Poststorage leucocyte reduction also has limited effectiveness in preventing FNHTR to platelets, whereas prestorage leucocyte reduction is highly effective. In contrast, both post- and prestorage leucocyte reduction are effective in preventing most FNHTR to red cells [5].

- Intravenous immunoglobulin (IvIg) is associated with adverse events such as headache and flu-like symptoms. Headache occurs in up half of patients treated with IvIg. Haemolytic transfusion reactions can also occur due to the presence of anti-A and Anti-B in IvIg products.

Was Fever Present?

- Fever is a common finding in most types of reactions. However, it does not occur in allergic transfusion reactions or with anaphylaxis. Therefore, fever can be useful to help differentiate between severe hypotension caused by bacterial contamination, acute haemolysis or TRALI (fever may be present) versus hypotension caused by anaphylactic shock (fever is absent).
- Was the rise in temperature ≥ 2 °C? Significant temperature increases are typically seen with bacterial contamination, especially if the patient has not been premedicated with an antipyretic or is not receiving antibiotic therapy. Increases in temperature greater than 2 °C are not usually seen with other types of reactions [6].

Volume of Component Transfused

The volume of the component transfused can also be an important consideration for a differential diagnosis.

- Some types of reactions are dose dependent; hence, they tend to occur towards the end of the transfusion after most of the component has been given. Such reactions include allergic reactions, FNHTR and TRALI. This observation becomes less useful when symptoms occur during the transfusion of multiple blood components. In this situation, it is difficult to determine whether the reaction is caused by the first unit transfused or the current unit that is being administered.
- Anaphylactic reactions can present after a small amount of component is transfused (1–10 mL) [7].
- Acute haemolytic reactions usually require at least 50–100 mL of red cells to be transfused before symptoms appear.

Other Considerations

- Always remember that the patient's clinical co-morbidities and therapies could also be causing many of the symptoms typical of acute transfusion reactions. Hence, these always need to be considered as part of the differential diagnosis.
- Although most reactions are relatively infrequent, it is possible for a patient to have more than one type of reaction concurrently. This possibility should always be considered when the patient presents with atypical findings.
- For many reaction types, there is a spectrum of severity, ranging from mild to severe, depending on such factors as the characteristics of the patient and blood component, and the amount of blood transfused. For example, bacterial contamination of a blood component may result in an acute septic reaction with high fever and hypotension. Alternatively, such a component may cause no or only mild symptoms.
- Consider how well you know the patient and their previous response to blood component transfusions. Less concern may be appropriate for a patient who develops hives every time they are transfused, whereas action would be appropriate for the sudden development of moderate respiratory symptoms in the multitransfused patient who has previously had no adverse events.

General Approach for Investigation and Treatment of Acute Transfusion Reactions

Using all the information noted above, the clinician must make a decision whether to stop the administration of the blood component temporarily or discontinue the transfusion, and must decide the extent of the investigations to be performed. Stopping and investigating every transfusion reaction is often assumed to provide the highest level of safety for the patient, but in reality may contribute to other morbidities, such as bleeding or respiratory/cardiovascular morbidity if an essential transfusion is delayed. Hence, some clinical judgement is required to ensure a balance between risk and benefit. The following approach should be used if there is any concern about patient safety and an investigation is required.

Action to Be Taken on the Clinical Unit

- Stop the transfusion immediately. The severity of some reactions is dose dependent. For example, the risk of severe morbidity and mortality with acute haemolysis is generally proportional to the volume of component transfused.
- Keep the intravenous (IV) line open with saline (or other appropriate IV solution) in case a decision is made to continue the transfusion or the patient requires other IV therapy.
- Support the patient's clinical symptoms with appropriate medical therapy.
- Perform a bedside clerical check to ensure that the name on the blood component and requisition matches the patient's armband/identifier.
- Look carefully at the remaining blood component to determine if there is any evidence of haemolysis or particulate matter. A contaminated unit of red cells may have discoloration either in the primary bag or in the first few segments closest to the blood bag.
- Complete a transfusion reaction form and notify the blood transfusion laboratory that a reaction has occurred. The transfusion laboratory will perform relevant investigations, notify the blood supplier if applicable so appropriate actions can be taken, such as recall of co-components, particularly in cases of TRALI or bacterial contamination, and ensure that relevant reactions are reported to the country's haemovigilance system (see Chapter 20). This reporting provides cumulative statistics about reactions that may be the first clue to a new emerging threat to the blood supply or a problem with component manufacturing.
- If a decision is made to perform a more extensive investigation to rule out problems with a donor unit (e.g. serological incompatibility causing haemolysis, bacterial contamination, TRALI), the remainder of the blood bag should be returned to the blood transfusion laboratory and/or blood service for further testing. Local policies should be followed for additional patient samples to be collected for specialised testing.

Action to Be Taken in the Laboratory

When a reaction is reported to the blood transfusion laboratory, a clerical check should always be performed to verify that the paperwork is accurate and that the correct component was issued for transfusion. To rule out haemolysis from the differential diagnosis, the following screening tests should be performed:

- Clerical check as mentioned.
- Centrifuge a posttransfusion sample of the patient's blood and observe the plasma for visual evidence of haemolysis.
- A direct antiglobulin test on a posttransfusion ethylenediaminetetraacetic acid (EDTA) sample taken from the patient.

If the clerical check does not indicate any problems and the two screening tests are negative, acute haemolysis as the cause of the reaction can usually be eliminated. However, if the patient's symptoms are severe and consistent with a haemolytic reaction, a complete serological workup may be indicated, including repeating the compatibility

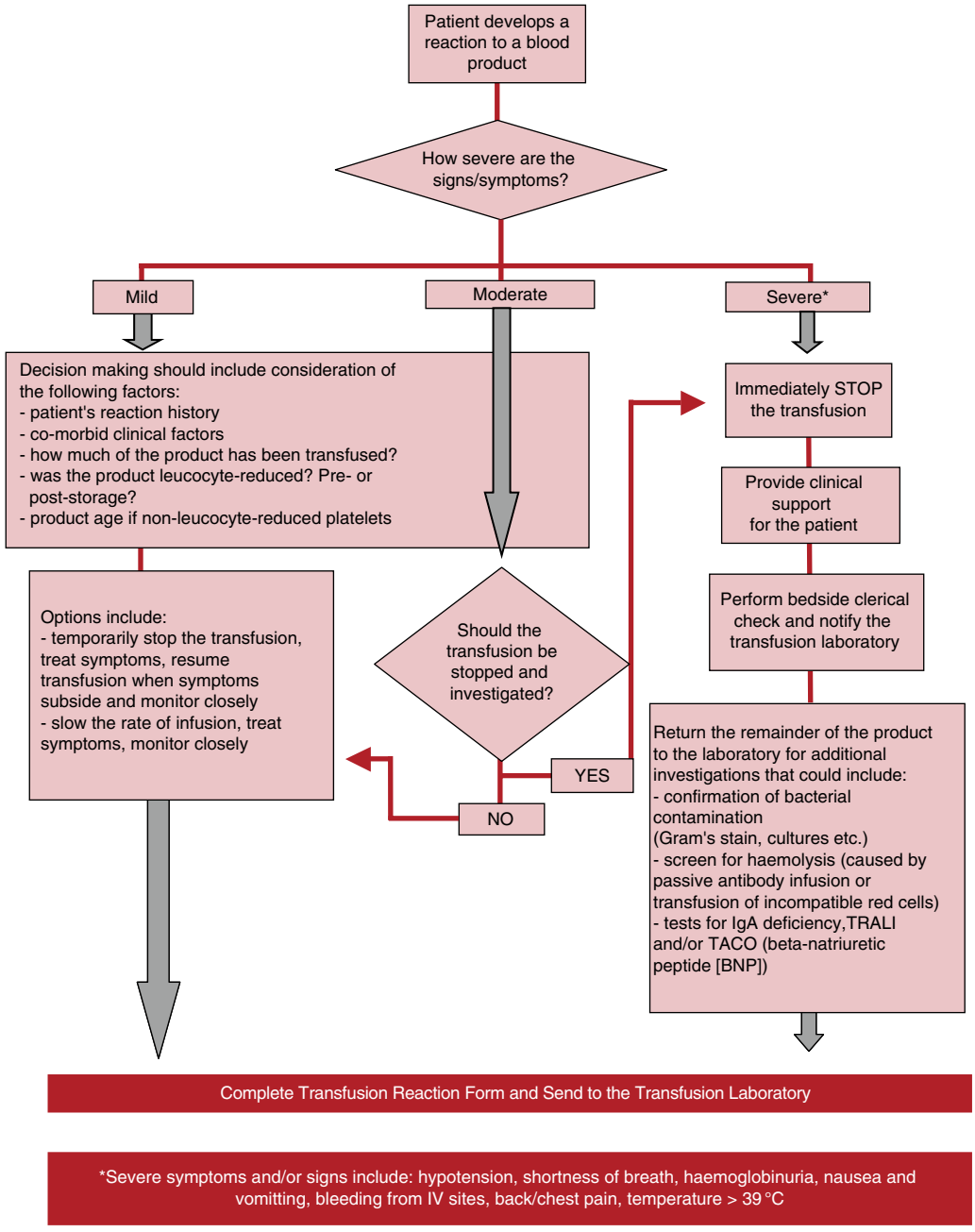

Figure 7.1 Flow diagram illustrating a possible approach for the management and investigation of an acute transfusion reaction. IgA, immunoglobulin A; TACO, transfusion-associated circulatory overload; TRALI, transfusion-related acute lung injury.

test on both the pre- and posttransfusion patient samples and specific tests for haemolysis (i.e. lactate dehydrogenase, haptoglobin, methaemalbumin).

All blood transfusion laboratories should have specific protocols for the investigation of other types of reactions. The Public Health Agency of Canada has developed guidelines for the investigation of suspected reactions caused by bacterial contamination, which can be accessed from the website https://www.canada.ca/en/public-health.html [8]. Similar documents may be available in other countries. Investigation of TRALI, anaphylaxis and TACO requires specialised testing, which may be available only from a reference centre or specialised laboratory [9–11]. However, each facility should have policies and procedures in place to direct and facilitate these investigations. Results from these specialised tests are not usually available in a timely manner. Hence, treatment and prevention strategies must be drawn up based on clinical findings and test results available on site.

Algorithm

As mentioned previously, some clinical judgement is required when deciding which reactions to investigate more fully and the management strategies required. Aggressive investigation of mild reactions can burden resources within the healthcare setting and may cause unnecessary delays in transfusion therapy for a patient in critical need of blood components. In contrast, patient safety should always be paramount. The algorithm in Figure 7.1 can be used as a guide to develop a safe but logical approach to managing acute transfusion reactions.

KEY POINTS

1) Decisions related to the investigation of acute transfusion reactions require some clinical judgement based on the severity of the reactions.
2) Effective management decision making requires that healthcare professionals understand the types of acute transfusion reactions that can occur and their pathophysiology.
3) Patient factors to consider when formulating the differential diagnosis include the history of transfusion, pregnancy, medications, previous reactions, types of symptoms and diagnosis and clinical co-morbidities.
4) Component factors to consider when formulating the differential diagnosis include the type of component, leucocyte reduction status, volume transfused and component age.
5) Each institution must have policies and procedures for the investigation of acute reactions.

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8 Haemolytic Transfusion Reactions

Edwin J. Massey[1], Robertson D. Davenport[2] and Richard M. Kaufman[3]

[1] *Velindre University NHS Trust and Cwm Taf Morgannwg University Health Board, Talbot Green, Wales, UK*
[2] *University of Michigan Health System, Ann Arbor, MI, USA*
[3] *Brigham and Women's Hospital, Boston, MA, USA*

Definition of a Haemolytic Transfusion Reaction

A haemolytic transfusion reaction (HTR) is the occurrence of lysis or accelerated clearance of red cells in a recipient of a blood transfusion. With few exceptions, these reactions are caused by immunological incompatibility between the blood donor and the recipient [1].

Haemolytic transfusion reactions are usually classified with respect to the time of their occurrence following the transfusion, but may also be classified on the pathophysiological basis of the site of red cell destruction, intravascular or extravascular. Finally, the term hyperhaemolysis is used, which, depending on the context, may indicate either the severity of the haemolysis or the pathophysiology underlying it (phagocytosis). The classification used by the Serious Hazards of Transfusion (SHOT) haemovigilance scheme in the UK is as follows [2]:

- *Acute HTRs (AHTRs)* are characterised by fever, a fall in haemoglobin (Hb), rise in bilirubin and lactate dehydrogenase (LDH) and a positive direct antiglobulin test (DAT). They generally present within 24 hours of transfusion.
- *Delayed HTRs (DHTRs)* occur more than 24 hours following a transfusion and are associated with a fall in Hb or failure to increment, a rise in bilirubin and LDH and an incompatible crossmatch not detectable pre transfusion.
- *Hyperhaemolysis* is characterised by more severe haemolysis than DHTR, with haemolysis affecting the transfused red cells and the patient's own red cells; there is a decrease in Hb to below pretransfusion levels, which is often associated with a reticulocytopenia. It may be triggered by a new red cell alloantibody, but frequently no new red cell antibody is identified. Hyperhaemolysis can be divided into acute and delayed hyperhaemolysis.

In the USA, the Centers for Disease Control and Prevention (CDC) have detailed definitions for HTRs, which may be classified as definitive, probable or possible. 'Definitive' HTRs fulfil the following criteria [3]:

- An *AHTR* occurs during, or within, 24 hours of cessation of transfusion with new onset of **any** of the following signs/symptoms: back/flank pain; chills/rigors; disseminated intravascular coagulation (DIC); epistaxis; fever; haematuria (gross visual haemolysis); hypotension; oliguria/anuria; pain and/or oozing

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at intravenous (IV) site; renal failure **and** two or more of the following: decreased fibrinogen; decreased haptoglobin; elevated bilirubin; elevated LDH; haemoglobinaemia; haemoglobinuria; plasma discoloration consistent with haemolysis; spherocytes on blood film **and either (immune mediated)** positive DAT for anti-IgG or anti-C3 **and** positive elution test with alloantibody present on the transfused red blood cells **or (non-immune mediated)** serological testing is negative, and physical cause (e.g. thermal, osmotic, mechanical, chemical) is confirmed.

- A *DHTR* is defined as a positive DAT for antibodies that developed between 24 hours and 28 days after cessation of transfusion **and either** a positive elution test with alloantibody present on the transfused red blood cells **or** a newly identified red blood cell alloantibody in recipient serum **and either** an inadequate rise of posttransfusion haemoglobin level or a rapid fall in haemoglobin back to pretransfusion levels **or** the otherwise unexplained appearance of spherocytes.
- The CDC does not have a separate category for hyperhaemolysis, but does have another entity entirely, described as a delayed serological transfusion reaction (DSTR), where laboratory tests indicate that the patient has formed antibodies against the donor(s) red cells but there is no clinical or biochemical evidence of haemolysis.

In general, with some exceptions, intravascular haemolysis is seen in AHTRs and extravascular haemolysis in DHTRs. During intravascular haemolysis, the destroyed red cells release free haemoglobin and other red cell contents directly into the intravascular space. These reactions are characterised by gross haemoglobinaemia and haemoglobinuria, which can potentially precipitate renal and other organ failure.

During extravascular haemolysis, red cells are removed from circulation primarily by the spleen. In these reactions the only feature may be a fall in Hb, but clinically significant DHTRs can occur, which may contribute to morbidity and even mortality in patients who are otherwise compromised by single or multiple organ failure prior to the reaction.

Pathophysiology of Haemolytic Transfusion Reactions

Antibody-Mediated haemolysis

There are three phases involved in antibody-mediated haemolysis, both acute and delayed (Figure 8.1):

- Antibody binding to red cell antigens, which may involve complement activation.
- Opsonised red cells interacting with and activating phagocytes.
- Production of inflammatory mediators.

Antigen–Antibody Interactions

Where an immunological incompatibility is responsible, the course of the reaction depends upon:

- The class and subclass (in the case of immunoglobulin [Ig]G) of the antibody.
- The blood group specificity of the antibody.
- The thermal range of the antibody.
- The number, density and spatial arrangement of the red cell antigen sites.
- The ability of the antibody to activate complement.
- The concentration of antibody in the plasma.
- The amount of red cells transfused.

Characteristics of the Antibody and Antigen

- The characteristics of the antibody (such as immunoglobulin class, specificity and thermal range) and those of the antigen sites against which antibody activity is directed (such as site density and spatial arrangement) are interrelated. Antibodies of a certain specificity, from different individuals, are often found only within a

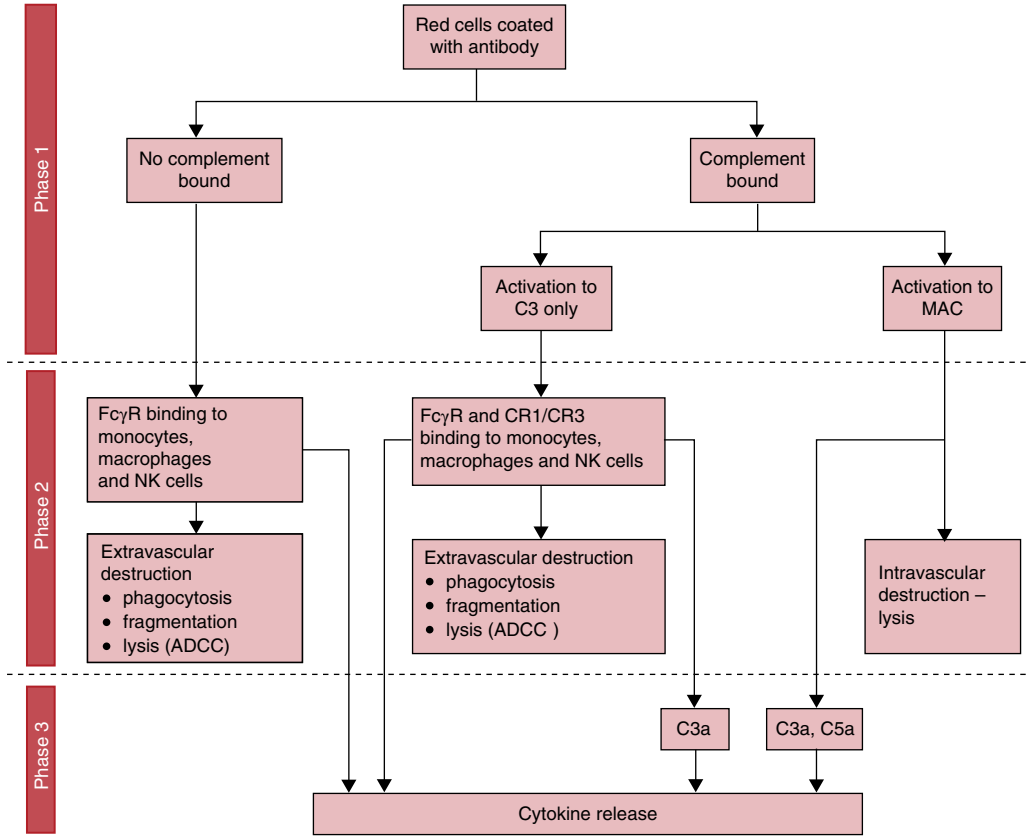

Figure 8.1 Pathophysiology of the haemolytic transfusion reaction (HTR). ADCC, antibody-dependent cell-mediated cytotoxicity; MAC, membrane attack complex; NK, natural killer.

particular immunoglobulin class and have similar thermal characteristics; in addition, red cells of a certain blood group phenotype, from different individuals, tend to be relatively homogeneous regarding the attributes of the relevant antigen. So, knowledge of the specificity of an antibody can help predict its clinical significance [4]. Three examples illustrate this concept.

- Anti-A, anti-B and anti-A,B antibodies are regularly present in moderate to high titre in the plasma of group O persons. These 'naturally occurring' antibodies are often both IgM and IgG, having a broad thermal range up to 37 °C, and are often strongly complement binding. The A and B antigens are present in large numbers (e.g. up to 1.2×10^6 A_1 antigen sites per cell) and are strongly *immunogenic* (provoking an immune response in an individual lacking the antigen). If a recipient with anti-A, anti-B or anti-A,B is transfused with donor red cells that express the cognate antigen (i.e. A and/or B), an AHTR is highly likely and may be fatal. Group O donor plasma (200–300 mL in platelets or plasma) can also cause haemolysis of the recipient's red cells if they are A, AB or B.
- Anti-Jk^a antibodies may be produced following immunisation of a Jk(a–) person. They are usually IgG (but may include IgM), are active at 37 °C and may bind complement. Jk(a+b–) red cells express only 1.4×10^4 Jk^a antigens per cell. Jk^a antibodies are sometimes difficult to detect because of the low titre of antibody. Anti-Jka characteristically evanesces over time; consequently, Jk(a+) blood may be

inadvertently transfused to patients with preexisting anti-Jk^a. These antibodies are frequently implicated in DHTRs.

- Anti-Lu^a antibodies may be produced following the immunisation of a Lu(a–) person or may be 'naturally occurring'. They are usually IgM (but often include IgA and IgG), sometimes react at 37 °C and are not usually complement binding. Lu^a antigens are poorly immunogenic. The antibody may not be detected in pretransfusion testing, because screening cells are usually Lu^a negative and because antibody levels fall after immunisation. Anti-Lu^a antibodies have not been implicated in AHTRs and have only rarely been implicated in DHTRs, which are usually mild.

Complement Activation

Antibody-mediated intravascular haemolysis is caused by sequential binding of complement components (C1–C9) on the red cell membrane. IgM alloantibodies are more efficient activators of C1 than IgG antibodies, since the latter must be sufficiently close together on the red cell surface to be bridged by C1q to activate complement. Activation to the C5 stage leads to release of C5a into the plasma and assembly of the remaining components of the membrane attack complex (MAC) on the red cell surface, leading to lysis.

Extravascular haemolysis is caused by non-complement-binding IgG antibodies or those that bind sub-lytic amounts of complement. IgG subclasses differ in their ability to bind complement, with the following order of reactivity:

$$IgG3 > IgG1 > IgG2 > IgG4$$

Activation of C3 leads to the release of C3a into the plasma and to C3b and iC3b deposition on red cells, promoting binding of the red cell to two complement receptors, CR1 (CD35) and CR3 (CD11b), which are both expressed on macrophages and monocytes. Hence, C3b and iC3b augment macrophage-mediated clearance of IgG-coated cells, and antibodies binding sub-lytic amounts of complement (e.g. Duffy and Kidd antibodies) often cause more rapid red cell clearance and more marked symptoms than non-complement-binding antibodies (e.g. Rh antibodies).

C3a and C5a are anaphylatoxins with potent pro-inflammatory effects, including oxygen radical production, granule enzyme release from mast cells and granulocytes, nitric oxide production and cytokine production [5].

Fc Receptor Interactions

IgG alloantibodies bound to red cell antigens interact with phagocytes through Fc receptors. The affinity of Fc receptors for IgG subclasses varies, with most efficient binding to IgG1 and IgG3. After attachment to phagocytes, the red cells are either engulfed or lysed through antibody-dependent cell-mediated cytotoxicity (ADCC).

Cytokines

Cytokines are generated during an HTR because of both anaphylatoxin generation (C3a, C5a) and phagocyte Fc receptor interaction with red cell-bound IgG. Some biological actions of cytokines implicated in HTRs are given in Table 8.1.

ABO incompatibility stimulates the rapid release of high levels of tumour necrosis factor (TNF)-α into the plasma, followed by CXCL-8 (interleukin [IL]-8) and CCL-2 (monocyte chemotactic protein [MCP]-1). In IgG-mediated haemolysis, TNF-α is produced at a lower level together with IL-1β and IL-6. CXCL-8 production follows a similar time course to that in ABO incompatibility.

IgG-mediated haemolysis, as opposed to IgM/complement-mediated haemolysis, also results in the production of the IL-1 receptor antagonist IL-1ra. The relative balance of IL-1 and IL-1ra may also, at least in part, account for some of the clinical differences between intravascular and extravascular haemolysis [6].

Table 8.1 Cytokines implicated in haemolytic transfusion reactions.

Terminology	Biological activity
Pro-inflammatory cytokines	
TNF, IL-1	Fever Hypotension, shock, death Mobilisation of leucocytes from marrow Activation of T and B cells Induction of cytokines (IL-1, IL-6, CXCL-8, TNF-α, CCL-2) Induction of adhesion molecules
IL-6	Fever Acute-phase protein response B-cell antibody production T-cell activation
Chemokines	
CXCL-8	Chemotaxis of neutrophils Chemotaxis of lymphocytes Neutrophil activation Basophil histamine release
CCL-2	Chemotaxis of monocytes Induction of respiratory burst Induction of adhesion molecules Induction of IL-1
Anti-inflammatory cytokines	
IL-1ra	Competitive inhibition of IL-1 type I and II receptors

CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; IL, interleukin; TNF, tumour necrosis factor.

Antibody Specificities Associated with Haemolytic Transfusion Reactions

These are given, together with the site of red cell destruction, in Table 8.2. A helpful review paper on the clinical significance of red cell antibodies has been written by Daniels and colleagues, with an online updated version provided by NHS Blood and Transplant (NHSBT) in the UK [4].

The Pathophysiology of Hyperhaemolysis (aka Post-Transfusion Haemophagocytic Syndrome)

Red cell antibodies are undetectable in approximately half of reported cases and posttransfusion haemophagocytic syndrome (PTHS) develops despite transfusion of crossmatch-compatible red blood cells (RBCs). It has been hypothesised that activated macrophages act as the main drivers of red cell destruction through direct phagocytosis. This is supported by histologically demonstrated macrophage expansion and erythrophagocytosis associated with a raised serum ferritin and reticulocytopenia, as described in haemophagocytosis of hereditary and acquired aetiologies [7].

Acute Haemolytic Transfusion Reactions

Aetiology and Incidence

These reactions are caused by existing antibodies, in the recipient or donor plasma, which are directed against the respective red cell antigens of the other party. It is salutary to remember that in high-income countries

Table 8.2 Antibody specificities associated with haemolytic transfusion reactions.

Blood group system	Intravascular haemolysis	Extravascular haemolysis
ABO, H	A, B, H	
Rh		All
Kell	K	K, k, Kp^a, Kp^b, Js^a, Js^b
Kidd	Jk^a (Jk^b, Jk^3)	Jk^a, Jk^b, Jk^3
Duffy		Fy^a, Fy^b, Fy^3
MNS		M, S, s, U
Lutheran		Lu^b
Lewis	Le^a	
Cartwright		Yt^a
Vel	Vel	Vel
Colton		Co^a, Co^b
Dombrock		Do^a, Do^b

HTRs cause more morbidity and mortality than transfusion-transmitted infection. Incompatible transfusion can occur for the following reasons:

- *Clerical error*: while taking and labelling the sample, laboratory compatibility testing and blood allocation, collection of the blood component and/or during bedside checking at administration.
- *Undetected antibody*: for example Kidd (Jk) (see above).
- *Intentional provision of blood components* as the best available, lowest-risk choice when the 'perfect' blood component is not available, e.g. O D-negative red cells (expressing the c antigen homozygously) transfused in an emergency as 'universal' blood to a patient who subsequently proves to have anti-c.

The majority of AHTRs have historically been due to the transfusion of ABO-incompatible red cells, but they can also be due to plasma containing high titres of ABO haemolysins in platelets or, less commonly, plasma transfusions (classically group O donor plasma into a group A recipient). ABO-incompatible platelet administration is unlikely to cause a reaction and such transfusions are routinely used to optimise platelet stock (see below).

The SHOT confidential reporting scheme has shown that when the patient was transfused with a blood component or plasma product that did not meet the appropriate requirements or that was intended for another patient, the sites of primary error were clinical areas in approximately 65% of cases, hospital laboratories in 34% of cases and blood establishments in 1% of cases. Frequently multiple errors contribute to incorrect blood component transfusion (IBCT). Examples of reported errors from several series are given in Box 8.1. ABO-incompatible (ABOi) transfusions are often unrecognised or not reported, but two surveys have found a frequency of 1 in approximately 30 000 transfusions [8,9].

Not all ABO-incompatible transfusions cause morbidity and mortality; mortality is dependent on the volume transfused and is 25% in recipients receiving 1–2 units of blood, reaching 44% with more than 2 units. However, even 30 mL of group A cells given to a group O recipient can be fatal. Less frequently, Kell, Kidd and Duffy antibodies can be responsible, and the acute reaction is due to a failure to detect, or take account of, the

Box 8.1 Errors resulting in 'wrong blood' incidents

Prescription, sampling and request
Failure to identify correct recipient at sampling
Correct patient identity at sampling but incorrectly labelled sample
Selection of incompatible products in an emergency

Transfusion laboratory
Took a correctly identified sample and aliquoted it into an improperly labelled test tube for testing
Took a wrongly identified sample through testing
Tested the correct sample but misinterpreted the results
Tested the correct sample but recorded the results on the wrong record
Correctly tested the sample but labelled the wrong unit of blood as compatible for the patient
Incorrect serological reasoning, e.g. O-positive fresh frozen plasma (FFP) to non-O-positive recipient

Collection and delivery of the wrong component to the ward
Failure to check recipient identity with unit identity

Bedside administration error
Recipient identity checked through case notes or prescription chart and not wristband
Wristband absent or incorrect

red cell alloantibody in either the antibody screen or crossmatch.

Errors are a major cause of HTRs. In SHOT reports up to 2010, HTRs accounted for 501/8110 (6%) and IBCT 2837/8110 (35%) of errors reported. In 2020, HTRs and IBCTs accounted for 46/3214 (1.4%) and 323/3214 (10%) of reports, respectively. Data from 2016–20 showed that although there were 19 ABOi red cell transfusions, there were 1495 near-misses where an ABOi transfusion would have resulted if not for the recognition that the wrong blood was present in the sample tube (wrong blood in tube, WBIT). A 2012 recommendation that two samples from two separate venepunctures should be analysed prior to transfusion except in emergency situations has been widely implemented in the UK and has contributed to this improvement [2].

Nearly all deaths from IBCT are due to ABOi transfusions, with 27 deaths reported to SHOT in which IBCT was causal or contributory between 1996 and 2010 [10]. This declined substantially between 1996 and 2020.

There were nine reported incidents of ABO incompatibility in 2020, including one resulting from coronavirus convalescent plasma infusion. Administration errors accounted for most of the ABOi transfusions (5/9), with two errors at component selection and two at collection. There were no deaths. Morbidity and mortality following HTR are now more commonly due to antibodies other than ABO [2].

Similar findings have been noted in other countries; details of the incompatibilities resulting in deaths reported to the US Food and Drug Administration (FDA) between 2015 and 2019 are provided in Table 8.3 [11].

Symptoms and Signs

These may become apparent after only 20 mL of ABOi red cells. Initial clinical presentations include the following:

- Fever and/or chills.
- Pain at the infusion site, lower back/flanks, abdomen, chest or head.
- Hypotension and/or tachycardia.
- Agitation, distress and confusion, particularly in the elderly.
- Nausea or vomiting.
- Dyspnoea.
- Flushing.
- Haemoglobinuria.

Table 8.3 Fatal haemolytic transfusion reactions reported to the Food and Drug Administration by implicated antibody, 2015–19.

Antibody	2015	2016	2017	2018	2019	Total
ABO	2	4	1	2	4	13
Multiple antibodies*	2	–	1	–	1	4
Other**	1	–	2	2	2	7
D	–	–	–	–	1	1
c	1	1	–	–	–	2
e	–	–	1	–	–	1
f	–	–	–	–	1	1
V	–	–	–	–	1	1
K	–	–	–	–	1	1
Fy^a	–	–	1	1	1	2
Jk^a	–	–	–	–	1	1
Jk^b	–	–	–	1	–	1
Jk^3	–	–	–	–	1	1
M	–	–	–	–	1	1
U	–	–	1	–	–	1
Wr^a	–	–	–	1	–	1
Total	6	5	7	7	11	33

*Multiple antibodies: In 2015 antibody combinations included E+K+Jka+M+Cob+Cw **and** C+E+S+Jkb+Fya+Fyb. In 2017 antibody combinations included Jka+M. In 2019 antibody combinations included Fya+Jkb.
**Other: 2015 included one report of hyperhaemolysis syndrome in which no new or additional antibody was identified. 2017 includes one report of hyperhaemolysis syndrome in which no new or additional antibody was identified, and one case of a haemolytic transfusion reaction where no new or additional antibody was identified. In 2018: 1) The case with anti-Jk^b also demonstrated anti-S and a hyperhaemolysis syndrome; 2) A case of transfused cold autoimmune haemolytic anaemia. 2019 included: 1) likely warm autoimmune haemolytic anaemia; and 2) a haemolytic transfusion reaction with no definitive serological findings.

In anaesthetised patients, the only signs may be uncontrollable hypotension, red urine due to haemoglobinuria, or excessive bleeding from the operative site, as a result of DIC.

Some of these symptoms and signs can also be features of other transfusion reactions, including febrile non-haemolytic reactions, allergic reactions, transfusion-related acute lung injury and bacterial contamination of the unit (see Chapters 9, 10, 11 and 18).

Complications

Acute kidney injury develops in up to 36% of patients with AHTR from acute tubular necrosis, induced by free haemoglobin and hypotension. Thrombus formation in renal arterioles may also cause cortical infarcts.

DIC develops in up to 10% of patients. TNF-α can induce tissue factor expression by endothelial cells and, together with IL-1, can reduce the endothelial expression of thrombomodulin. Thromboplastic material is also liberated from leucocytes by complement activation [6].

Table 8.4 Immediate medical management of an acute transfusion reaction.

Symptoms/signs	Likely diagnosis	Actions
Isolated fever or fever and shivering, stable observations, correct unit given	FNHTR	Paracetamol 1 g orally/PO (USA: acetaminophen 625 mg), continue transfusion slowly observations of P, BP and T every 15 min for 1 h, then hourly. If no improvement, then call haematology medical staff
Pruritus and/or urticaria	Allergic transfusion reaction	Chlorpheniramine 10 mg IV (USA: diphenhydramine 25–50 mg PO or IV) and other actions as for suspected FNHTR
Any other symptoms/signs, hypotension or incorrect unit	Assume to be an acute haemolytic transfusion reaction in first instance	Discontinue transfusion, normal saline to maintain urine output > 1 mL/kg/h. Full and continuous monitoring of vital signs. Call haematology medical and transfusion laboratory staff immediately for further advice/action. Send discontinued unit of blood with attached giving set and other empty packs, after clamping securely, to the transfusion laboratory

BP, blood pressure; FNHTR, febrile non-haemolytic transfusion reaction; IV, intravenous; P, pulse; PO, per os; T, temperature.

Immediate Management of Suspected Acute Haemolytic Transfusion Reactions

See Chapter 7 and British Society for Haematology guidelines [12].

Actions for Nursing Staff

If a patient develops symptoms or signs suggestive of an acute transfusion reaction mentioned above, the nursing staff should:

- Stop the transfusion, leaving the infusion line or giving set attached to the blood pack.
- Use a new giving set and keep an IV infusion running with normal saline.
- Call a member of the medical staff.
- Check that the patient identity as provided on the wristband corresponds with that given on the label on the blood pack and on the compatibility form.
- Save any urine to test for haemoglobinuria.
- Monitor the patient's pulse (P), blood pressure (BP) and temperature (T) at 15-minute intervals.

Actions for Medical Staff

The immediate actions depend upon the presenting symptoms and signs. These are summarised in Table 8.4.

Investigation of Suspected Acute Haemolytic Transfusion Reactions

Blood samples should be taken from a site other than the infusion site for the investigations listed in Table 8.5.

Other Reactions Characterised by Haemolysis

In patients with autoimmune haemolytic anaemia, transfusion may exacerbate the haemolysis and be associated with haemoglobinuria.

Donor units of red cells may also be haemolysed by:

- Bacterial contamination.
- Excessive warming.
- Erroneous freezing.
- Addition of drugs or IV fluids.

Table 8.5 Laboratory investigation of a suspected acute haemolytic transfusion reaction.

Blood test	Rationale/findings
Full blood count	Baseline parameters, red cell agglutinates on film
Plasma/urinary haemoglobin	Evidence of intravascular haemolysis
Haptoglobin, bilirubin, lactate dehydrogenase	Evidence of intravascular or extravascular haemolysis
Blood group	Comparison of posttransfusion and retested pretransfusion samples, to detect ABO error not apparent at bedside. Unexpected ABO antibodies post transfusion may result from transfused incompatible plasma. The donor ABO group should be confirmed
Direct antiglobulin test (DAT)	Positive in majority, pretransfusion sample should be tested for comparison. May be negative if all incompatible cells destroyed
Compatibility testing	An indirect antiglobulin test (IAT) antibody screen and IAT crossmatch using the pre- and posttransfusion samples provide evidence for the presence of alloantibody. Elution of antibody from posttransfusion red cells may aid in identification of antibody or confirm specificities identified in serum in cases of non-ABO incompatibility. Red cell phenotype should also be performed on recipient pretransfusion sample and unit in cases of non-ABO incompatibility, to confirm absence in patient and presence in unit of corresponding antigen
Urea/creatinine and electrolytes	Baseline renal function
Coagulation screen	Detection of incipient disseminated intravascular coagulation
Blood cultures from the patient and implicated pack(s)	In event of septic reaction caused by bacterial contamination of unit, which may be suspected from inspection of pack for lysis, altered colour or clots

- Trauma from extracorporeal devices.
- Red cell enzyme deficiency.

Management of a Confirmed Acute Haemolytic Transfusion Reaction

The management of HTRs should be determined by the severity of the clinical manifestations:

- Maintain adequate renal perfusion while avoiding volume overload by:
  - Maintenance of circulating volume with crystalloid and/or colloid infusions.
  - If necessary, inotropic support.
- Transfer to a high-dependency area where continuous monitoring can take place.
- Repeat coagulation and biochemistry tests 2–4-hourly.
- If urinary output cannot be maintained at 1 mL/kg/h, seek expert renal advice.
- Haemofiltration or dialysis may be required for the acute tubular necrosis. If DIC develops, blood component therapy may be required. After investigation of the incompatibility causing the AHTR, urgent transfusion of compatible blood may be required.

Prevention of Acute Haemolytic Transfusion Reactions

Prevention of 'Wrong Blood' Incidents

- Prevention of the multiplicity of errors that can contribute to the transfusion of ABOi red cells requires an effective quality system for the entire process, which will involve:
  - Adherence to national guidelines and standards.
  - Local procedures that are agreed, documented and validated.
  - Training and retraining of key staff.
  - Regular error analysis and review.
  - Reporting to local risk management/assurance committee.
  - Reporting to regulatory bodies such as the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK, the FDA in the USA and to national haemovigilance schemes to contribute to the understanding of the extent and underlying causes.

These aspects are specifically covered in Chapter 23.

- Since most errors leading to an ABOi transfusion are due to misidentification of the patient or patient's sample, unique patient identifiers must be used diligently throughout the hospital and laboratory [2,13,14].
- Access to previous transfusion records containing historical ABO groups should be available at all times.
- Ideally computerised systems should verify details of the patient, the sample taken for compatibility testing and the unit of blood at all steps, including at the bedside.

Prevention of Non-ABO Acute Haemolytic Transfusion Reactions

- For recurrently transfused patients, attention must paid to the interval between sampling and transfusion, to enable detection of developing antibodies. In the UK, for patients transfused within the previous 14 days, the pretransfusion sample should be within three days of the next transfusion [14]. In the USA, the pretransfusion sample must be taken within three days if the patient has been transfused or pregnant within the past three months. Similar requirements exist in other countries.
- In the presence of multiple red cell alloantibodies, when compatible red cells are unavailable in an emergency, IV immunoglobulin (e.g. 1 g/kg as a one-off dose potentially repeated for a second day) and/or steroids (e.g. oral prednisolone 2 mg /kg to a maximum dose of 60 mg daily with tapering; or methylprednisolone 1 g as a one-off dose potentially repeated; or hydrocortisone 100 mg six-hourly if methylprednisolone is not readily available and the patient is unable to take oral steroid) have been used, with anecdotal reports of ameliorating a potential haemolytic or 'hyperhaemolytic' episode (see below). Other immunosuppressant/immunomodulatory drugs have been used including eculizumab, a monoclonal antibody blocking the cleavage of complement component C5 [15].

Delayed Haemolytic Transfusion Reactions

Aetiology and Incidence

With few exceptions, DHTRs are due to secondary immune responses following reexposure to a given red cell antigen, so there is a rapid increase in the alloantibody concentration and destruction of red cells.

- The antibodies most commonly implicated are directed against Kidd blood group antigens, followed by the Rh, Duffy and Kell antigens. In around 10% of reported cases, more than one alloantibody was present [2,16].

- Frequently, there are no clinical signs of red cell destruction, but subsequent investigations reveal a positive DAT and the emergence of a red cell antibody. This situation has been termed a delayed serological transfusion reaction (DSTR) [16].
- Kidd and Duffy antibodies are more likely to cause symptoms and be associated with a DHTR rather than a DSTR.
- Estimates of the frequency of DHTR and DSTR vary, but in a series reported from the Mayo Clinic, the frequency of DHTR was 1 in 5405 units and of DSTR was 1 in 2990 units, giving a combined frequency of 1 in 1899 units transfused [17].
- DHTRs are in themselves rarely fatal, but can contribute to death in patients with significant co-morbidities.
- Of transfusion fatalities reported to the FDA between 2010 and 2014, 21% (38) were due to HTR; of these, 34% (13/38) were due to ABO antibodies and in 18% (7/38) more than one alloantibody was present in the serum. The most frequent single specificities were Jk^a or Jk^b, which constituted 13% (5/38) [11].
- In 2020, 0.78% (25/3214) of reports to SHOT were DHTRs [2] featuring a disproportionate number of patients with sickle cell disease.

Signs and Symptoms

These usually appear within 5–10 days following the transfusion, with a range of 1–41 days. The onset of haemolysis may be insidious and only evident from results of posttransfusion samples. The most common features are:

- Fever.
- Fall in haemoglobin concentration.
- Jaundice and hyperbilirubinaemia.

Hypotension and kidney injury are uncommon (6% of cases). In the postoperative period in particular, the diagnosis may be overlooked, and the symptoms and signs incorrectly attributed to continuing haemorrhage or sepsis. In the setting of sickle cell disease, DHTR can be particularly severe (see below).

Management

The majority of DHTRs require no treatment, because red cell destruction occurs gradually as antibody synthesis increases. Haemolysis may, however, contribute to the development of life-threatening anaemia, particularly in patients with ongoing bleeding, and urgent investigations are often required to provide antigen-negative units.

Expert medical advice may be required for treatment of the hypotension and renal failure. When accompanied by circulatory instability and renal insufficiency, a red cell exchange transfusion with antigen-negative units can curtail haemolysis. Future transfusions of red cells should be negative for the respective antigen.

Investigation of Suspected Delayed Haemolytic Transfusion Reactions (see Chapter 7)

- The peripheral blood film is likely to show spherocytosis. Other evidence of haemolysis – namely hyperbilirubinaemia, elevated LDH, reduced serum haptoglobin, haemoglobinaemia, haemoglobinuria and haemosiderinuria – is useful to confirm the nature of the reaction and to monitor progress.
- The DAT usually becomes positive within a few days of the transfusion, until the incompatible cells have been eliminated.
- Further serological testing on pre- and posttransfusion samples should be undertaken in accordance with the schedule provided for AHTR.
- The antibody may not be initially apparent in the posttransfusion serum, but can be eluted from the red cells. If the red cell eluate is inconclusive, then a repeat sample should be taken after 7–10 days, to allow for an increase in antibody titre. However, additional, more sensitive techniques may be needed to detect the antibody and may require the help of a reference laboratory.

- Since many cases have more than one alloantibody present, the panels used for antibody identification must have cells of appropriate phenotypes to exclude additional specificities.

Prevention

Previous transfusion records may disclose the presence of antibodies undetectable at the time of cross-matching, and all patients should be questioned regarding previous transfusions and pregnancies. Patients with a clinically significant red cell alloantibody should be provided with an antibody card to help ensure that they receive antigen-negative units for all future transfusions. When the care of patients requiring transfusion is shared between hospitals, there must be good communication between laboratories and clinical teams. In England, blood group and compatibility testing results performed in NHSBT via a software package to all hospitals enables the cross-checking of historical blood group and antibody information to minimise the risk of error.

Laboratories should ensure that their antibody screen is effective in detecting weak red cell alloantibodies and that screening cells are taken from homozygotes, where the corresponding antibodies are less easy to detect when red cells with heterozygous expression of the relevant antigen is used. Pretransfusion testing is covered in detail in Chapter 23.

Haemolysis Resulting from Haemopoietic Stem Cell Transplantation (see Chapter 41)

Poor communication between departments and geographical sites and associated errors has been a recurrent cause of IBCT and HTR following stem cell transplant and have been addressed by the recommendations 'Safe transfusions in haemopoietic stem cell transplant recipients', which are available at various web addresses in the UK, including the SHOT website [18].

Major ABO-Incompatible Transplants

Infusion of bone marrow during major ABOi transplants can result in an AHTR (the recipient has antibodies against the donor's red cells, e.g. group A donor, group O recipient). The risk is dependent on the antibody titre of the recipient and the volume of red cells in the marrow harvest. Peripheral blood stem cell products rarely have enough red cells to result in clinical AHTR, even if there is ABO incompatibility.

Minor ABO-Incompatible Transplants

Most patients transplanted with minor ABOi marrow (the donor has antibodies against the recipient's red cells, e.g. O donor, A recipient) develop a positive DAT, but only 10–15% of patients develop clinically significant haemolysis. Haemolysis in minor ABO incompatibility is short-lived and exchange transfusion is rarely required. Red cells and plasma-containing components (platelets, fresh frozen plasma [FFP] and cryoprecipitate) should be compatible with both recipient and donor.

It has been suggested that the use of peripheral blood stem cells may increase the risk of significant haemolysis, since the number of lymphocytes infused with the graft is increased, and three deaths due to an AHTR were reported between 1997 and 1999 in minor ABO-i transplants. Several cases due to anti-D have been described, and antibody production has persisted for up to one year [18,19].

Delayed Haemolysis Following Organ Transplantation (Passenger Lymphocyte Syndrome)

Donor-derived B lymphocytes within the transplanted organ may mount an anamnestic response against the recipient's red cell antigens. Donor-derived antibodies are usually directed against antigens within the ABO and Rh systems. If ABO-mismatched organs are transplanted, the frequency of occurrence of donor-derived antibodies and haemolysis increases with the lymphoid content

of the graft, from kidney to liver to heart–lung transplants. The figures for haemolysis are 9%, 29% and 70%, respectively. The frequency of haemolysis increases with an O donor and an A recipient. Pretransplant isohaemagglutinin titres do not appear to predict the incidence or severity of haemolysis.

The ABO antibodies, which appear 7–10 days after transplant, last for approximately one month. Haemolysis is usually mild, although several cases of renal failure and one death have been reported. It can be ameliorated by switching to group O cells, either at the end of surgery or postoperatively if the DAT becomes positive.

Rh antibodies have been described following kidney, liver and heart–lung transplants. They can cause haemolysis for up to six months, which may need treatment. Haemolysis occurs 7–10 days after transplantation, with an unpredictable and abrupt onset [20].

Haemophagocytic/Hyperhaemolytic Transfusion Reactions and Haemolytic Transfusion Reactions in Sickle Cell Disease

The frequency of alloimmunisation in sickle cell anaemia is dependent upon the nature and success of the extended red cell antigen-matching policy employed. Approximately 40% of patients who are alloimmunised have experienced or will experience a DHTR.

DHTRs can be responsible for major morbidity in sickle cell disease. The term 'sickle cell haemolytic transfusion reaction (SCHTR) syndrome' has been suggested to capture some of the distinctive features that accompany a reaction (see Chapter 30). A similar syndrome of hyperhaemolysis or hyperhaemolytic transfusion reaction (HHTR) has been described in other transfusion-dependent patients. The term post-transfusion haemophagocytic syndrome (PTHS) may give greater clarity to the specific pathophysiology and management by differentiating this syndrome from typical AHTR and DHTR. These features of hyperhaemolysis or PTHS are as follows:

- Deteriorating anaemia after transfusion, with evidence of haemolysis of autologous red cells (bystander immune haemolysis) as well as transfused red cells.
- Marked reticulocytopenia (relative to pretransfusion levels).
- Ferritin levels rising dramatically, as seen in haemophagocytic syndromes, and bone marrow aspirate showing haemophagocytosis of precursors and mature red cells. It is likely that erythroid precursors and reticulocytes are removed through mechanisms other than IgG and Fc receptors, such as the integrins α4β1 and VCAM-1.
- Symptoms suggestive of a sickle cell pain crisis that develop or are intensified during the HTR.
- Subsequent transfusions may further exacerbate the anaemia and it may become fatal.
- Some patients have multiple red blood cell alloantibodies and may also have autoantibodies, which make it difficult or impossible to find compatible units of RBCs.
- However, in other patients the DAT may be negative, no alloantibodies are identified, and serological studies may not provide an explanation for the HTR; even red cells that are phenotypically matched with multiple patient antigens may be haemolysed.

Management involves withholding further transfusion and treating with corticosteroids (methylprednisolone 1 g daily for three days), while intravenous immunoglobulin (IvIg, 1 g/kg/day for two days) may have been beneficial in some cases and other immunosuppressive agents, including the monoclonal antibodies tociluzimab blocking the inflammatory protein IL-6 and eculizumab inhibiting terminal complement activation at the C5 protein, have been used anecdotally [7,21,22].

It is recommended that patients with sickle cell disease are phenotyped prior to transfusion and that blood is matched for Rh c, C, D, e, E and K (see Chapter 34).

Acute Haemolysis from ABO-Incompatible Platelet Transfusions

Rarely, the passive transfusion of anti-A or anti-B present in a platelet pack will cause haemolysis in the recipient. This is mostly seen in type A recipients of type O platelets. Clinically significant reactions are rare: passive anti-A/B becomes diluted in the recipient's plasma and it will also bind to soluble or endothelial A or B antigens. The typical anti-A/B titre in a platelet donor is of the order of 1 : 128, but may sometimes exceed 10 000. Severe and even fatal AHTRs have been reported, where a large amount of incompatible ABO antibody is transfused into a recipient with a small plasma volume. In the UK, all platelet units must be screened for anti-A/B using a cut-off titre of 1 : 100. Packs from donors who have titres below this level are marked 'HT negative' or high titre negative. Approximately 10% of platelet units will have titres above 1 : 100 and these are only issued to ABO-identical recipients. In the USA, no preventive strategy is currently mandated and local practices vary. Association for the Advancement of Blood and Biotherapies (AABB)-accredited transfusion services are simply required to have a policy concerning the transfusion of products having significant amounts of incompatible ABO antibodies. It should be noted that in the UK at the time of writing pooled platelets are resuspended in platelet additive solution (PAS), which should significantly reduce this risk (and in some countries group O pooled platelets in PAS are used for all recipients without requiring HT testing). Apheresis platelets remain suspended in plasma in many countries.

KEY POINTS

1) Haemolytic transfusion reactions remain a common, often preventable cause of immediate morbidity and mortality following a transfusion internationally. However, other complications of transfusion are now more common, notably transfusion-associated circulatory overload (TACO), overtransfusion and, conversely, delays and undertransfusion.
2) The clinical presentations are diverse and they can be unrecognised or misdiagnosed.
3) Most fatal acute haemolytic transfusion reactions (AHTRs) have historically been due to the transfusion of ABO-incompatible (ABOi) red cells, but there is evidence that increased transfusion safety awareness has reduced the frequency of this. Other causes of AHTR are overtaking ABO in countries where haemovigilance schemes have been successful.
4) The transfusion of ABOi red cells is the result of an error occurring at any stage in the transfusion process. Patient identification errors are the most frequent culprit.
5) Post-transfusion haemophagocytic syndrome (less clearly described as hyperhaemolyis) can be independent of red cell alloantibodies and if accurately diagnosed may be amenable to a different therapeutic approach.
6) Devising and successfully implementing measures to overcome these preventable and fatal errors are a challenge, but should be a priority for those involved in transfusion practice.

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9 Febrile and Allergic Transfusion Reactions

Sarah K. Harm and Mark K. Fung

Department of Pathology and Laboratory Medicine, University of Vermont Medical Center, Burlington, VT, USA

Febrile non-haemolytic transfusion reactions (FNHTR) and allergic reactions are two of the most commonly encountered non-infectious complications of a blood product transfusion. The fever and/or chills of an FNHTR are a result of cytokine release from either donor or recipient white blood cells. Symptoms are usually self-limited and administration of leuco-reduced blood products can help prevent future reactions. Antipyretic administration may help decrease the fever, but premedication does not appear to reduce the incidence of FNHTRs. Allergic reactions are most commonly due to reactions against plasma proteins. Symptomatic relief of mild urticarial reactions can usually be achieved with the use of antihistamines, but premedication does not appear to reduce the incidence of allergic reactions. Although anaphylactic reactions are uncommon, close monitoring of patients during transfusions with a slow initial rate of transfusion permits prompt termination of the transfusion and treatment to minimise further morbidity or mortality.

Signs and symptoms typical of these reactions can also be associated with other types of transfusion reactions and/or caused by treatments and medications that the patient may be receiving as well as co-morbidities. Hence, establishing causation and an appropriate management strategy can be challenging. In this chapter, FNHTRs and both mild and severe forms of allergic reactions will be discussed.

Febrile Non-haemolytic Transfusion Reactions

Clinical Presentation

The classical definition of an FNHTR includes fever (usually defined as ≥ 1 °C rise in temperature) during or within four hours of completing the transfusion, along with other symptoms that can include a cold feeling, chills and a generalised feeling of discomfort or malaise. Less frequently, headache, nausea and vomiting may also occur, and, in severe reactions, rigors can be present. Although fever is a component of the classic definition, in practice only a minority of patients develop a fever and chills; cold and discomfort are the primary symptoms [1]. Therefore, the use of the International Society for Blood Transfusion (ISBT) or Centers for Disease Control and Prevention (CDC) haemovigilance definitions of an FNHTR, which include having either a ≥ 1 °C rise in temperature to 38 °C or greater or chills and/or rigors, may be more

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appropriate [2,3]. However, temperature rises as small as 0.6 °C may be relevant for products such as plasma [4]. Thus, an FNHTR can occur in with a temperature rise of < 1 °C or the absence of a fever.

Differential Diagnosis

Unfortunately, the signs and symptoms that are commonly associated with an FNHTR are not specific for an FNHTR. When a transfused patient develops fever or chills, the differential diagnosis should include:

- FNHTR.
- Acute haemolytic reactions.
- Delayed haemolytic reactions.
- Bacterial contamination.
- Transfusion-related acute lung injury (TRALI).
- Transfusion-associated circulatory overload (TACO).
- Acute pain reactions.
- Co-morbid conditions.
- Medications.

Although all these reactions can be associated with fever, it is especially important to rule out acute haemolysis, bacterial contamination and hypoxia (caused by TRALI or TACO) in view of their morbidity and mortality unless rapidly recognised and treated. More recently, TACO has been associated with inflammatory signs and symptoms in a subset of these reactions, and in two-thirds of these TACO–FNHTR cases, an inflammatory sign or symptom was the initial presentation [5]. Bacterial contamination and TRALI also have implications for donor management and handling of other components prepared from the implicated donation, emphasising the importance of considering these types of reactions in the differential diagnosis (see Chapters 11 and 18).

Long-term morbidity and mortality are not usually associated with FNHTRs; however, they cause the patient discomfort and distress and require healthcare resources for treatment and investigation. Preliminary data suggest that patients who experience FNHTR may be more likely to develop red cell alloimmunisation through modulation of the immune system towards a Th2 response to foreign red cell antigens [6], and highlight the potential importance of preventing an otherwise relatively minor transfusion reaction.

To further complicate the investigation of fever and chills in a transfusion recipient, many patient populations that require frequent transfusion have co-morbidities that cause similar symptoms, such as neutropenic fevers, medication-induced febrile reactions or postoperative temperature. Thus, a wide clinical perspective should be maintained during investigation of a febrile patients after a transfusion.

Frequency

The frequency of FNHTRs varies with the following:

- Patient population.
- Type of blood components being transfused.
- Age of the blood component.

Reactions to platelets occur more frequently than reactions to red cells. However, precise estimates of FNHTR associated with platelet transfusions are difficult, as milder reactions are likely to be underreported. In a general hospital population, FNHTRs to red cells occur with 0.04–0.44% of transfusions, while the frequency of reactions to platelets is higher, ranging from 0.06% to 2.2%. The rise in temperature for an FNHTR after red cell transfusions is much smaller than that for platelets, and this difference may account for some of the differences in the frequency of reactions with different types of blood components [4].

In some patient populations, such as adult haematology/oncology patients, reactions to platelets are even more common, occurring in up to 37% of transfusions of non-leucocyte-reduced platelets [7]. When prestorage leucocyte-reduced platelets in plasma are transfused, the frequency of

acute reactions decreases dramatically (< 2% of transfusions) [8]. The use of platelet additive solution (PAS) in prestorage leucocyte-reduced platelets has decreased the rate of FNHTR further to 0.17% [9]. In paediatric intensive care unit (ICU) patients, 1.6% of blood components transfused (40/2509) were associated with acute reactions, with FNHTR accounting for 60% (24/40) of these events [10]. Overall, paediatric patients are up to four times more likely to have an FNHTR than adult patients [11,12]. FNHTRs to blood components other than red cells and platelets are rare and there are limited data to estimate their frequency.

Pathogenesis

The pathogenesis of FNHTRs is multifactorial and varies for red cells and platelets. The current understanding of the mechanisms causing these reactions is summarised below.

Factors Associated with the Blood Component

- *Antibody mechanism*: patient's plasma contains a leucocyte antibody that reacts with leucocytes present in the blood product, resulting in the release of endogenous pyrogens and/or cytokines by the donor leucocytes, which act on the hypothalamus to cause fever. An antigen–antibody reaction is believed to be the primary mechanism causing FNHTRs to red cells, but probably accounts for less than 10% of FNHTRs to platelets [7]. Interestingly, the use of platelets that contain trace amounts of ABO-incompatible red blood cells (e.g. blood group O patient receiving group A platelets) that may generate a transient inflammatory reaction is not associated with an increased incidence of FNHTRs [13].
- *Leucocyte/platelet-derived biological response modifiers*: during storage of the blood product, pro-inflammatory cytokines interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α are released from leucocytes present in the blood component. This typically happens when the component is stored at room temperature. Cytokines accumulate to high levels during the product storage period and, when infused, cause fever by stimulation of the hypothalamus. This is the primary mechanism responsible for FNHTRs to platelets. Platelets are stored at room temperature and have high cytokine concentrations at the end of the storage period if leucocytes are present [14]. In addition, many platelet-derived cytokines, including CD40 ligand (sCD40L) and RANTES, also accumulate in stored platelets and may stimulate pro-inflammatory cytokines, including IL-6, IL-8 and monocyte chemotactic protein (MCP)-1. Multiple biological response mediators may act synergistically [15].
- *Other biological response modifiers (BRMs)*: other BRMs such as complement and neutrophil priming lipids have been detected in some stored blood products and it is hypothetically possible that they may cause or contribute to FNHTRs in some patients. However, there are no clinical data linking these substances to an increased risk of an adverse event [7].

Factors Associated with Patient Susceptibility

Patient factors may also play a role in susceptibility to FNHTRs. Reactions may be more common in females due to leucocyte antibody formation during pregnancy. Patients whose disease or treatment results in an inflammatory response experience reactions through the additive effect of transfusion-related and endogenous cytokines. Furthermore, genetic traits may cause an increase in inflammatory cytokine gene expression and an increased susceptibility to FNHTR, for example *1L1RN*2.2* [16].

Management of Febrile Non-haemolytic Transfusion Reactions

The management of FNHTRs includes the exclusion of other causes of fever, which can be difficult in patients with co-morbid

conditions. For example, a patient with neutropenic fevers from chemotherapy might also require transfusion, and fever after a transfusion may reflect a true FNHTR and/or the patient's underlying disease. So, the management strategy for FNHTRs requires clinical judgement and must balance the benefits and risks of investigation and treatment. The following questions should be considered:

- *Is the blood component leucocyte reduced?* The risk of an FNHTR in a setting where all red cell and platelet components are prestorage leucocyte reduced is low (see above). Here, stopping the transfusion and investigating every reaction not only consume significant healthcare resources, but also may delay transfusion of important components.
- *Does the patient have a history of FNHTRs?* Some patients are susceptible to repeated FNHTRs, for example because of the presence of leucocyte antibodies; hence the patient's history of reaction should be considered.
- *If a temperature increase occurred, was it greater than or equal to 2 °C?* It is uncommon for the temperature to rise more than 2 °C with an FNHTR. In this situation, bacterial contamination should be suspected, the transfusion should be stopped immediately and appropriate investigations initiated.
- *Would you describe the patient's signs and symptoms as mild, moderate or severe*? If the symptoms are mild, a less aggressive management approach may be initiated, but careful observation of the patient is essential. If the symptoms are severe, the transfusion should be stopped immediately and supportive care given to the patient. Investigations to rule out other possible causes of the reaction should be initiated. Moderate clinical findings require careful consideration and clinical judgement to decide how to proceed.

Finally, investigation of FNHTRs associated with red cell transfusions should exclude an acute haemolytic transfusion reaction. Haemolysis following platelet transfusion is rare, but can occur when the plasma of the platelet product contains an ABO antibody, usually high titre, that reacts with the patient's red cells.

The care of patient with an FNHTR should also aim to alleviate their signs and symptoms, including prescription of antipyretics and possibly temporary discontinuation of the transfusion. Medications should never be injected into the blood component. In most cases, the transfusion can be resumed once the signs and symptoms subside. There is some evidence that pethidine (or meperidine in North America) is an effective treatment for alleviating rigors associated with transfusions, although care should be taken in patients with compromised respiratory status and to avoid possible drug–drug interactions with pethidine or meperidine.

A conservative strategy for minimising the risk to patients while investigating reactions would include the following steps:

- Temporarily stop the transfusion but keep the line open with saline.
- Perform a bedside clerical check between the blood and the patient to ensure that the right blood has been transfused.
- Observe the blood product to determine whether there is discoloration or particulate matter present.
- Notify the blood transfusion laboratory and send appropriate samples if laboratory investigations are deemed necessary to rule out other causes of acute reactions with fever.

Prevention of Febrile Non-haemolytic Transfusion Reactions

As the pathogenesis of FNHTRs is different for red cells and platelet transfusions, the strategy for their prevention also depends on the blood component being transfused.

Red Cells

Since most reactions to red cells are caused by the leucocyte antigen–antibody mechanism, the primary way to prevent these reactions is to reduce the number of leucocytes in the red cell component. Prevention can be accomplished for most patients by removing approximately one log of leucocytes < 10^8 leucocytes/unit of red cells. This is readily achieved using current leucocyte-reduction filters that generate products with < 10^6 leucocytes, which is well below the threshold needed to prevent most FNHTRs. If a patient still reacts to a leucocyte-reduced red cell product, other options for preventing future reactions include washing and/or selecting fresher blood for transfusion [7]. Premedication with antipyretics can also be considered, but will only be effective against the febrile component of the reaction, with no effect on chills or rigors (see below for further discussion).

Platelets

Most platelet reactions (90%) are caused by leucocyte-derived cytokine accumulation during storage. So, FNHTRs to platelets can be prevented by prestorage leucocyte reduction by using filtration or centrifugation (buffy coat method of platelet preparation), but not by poststorage leucodepletion. If prestorage leucocyte-reduced platelets are not available, the plasma supernatant of the stored platelets can be removed and replaced with a suitable PAS or saline or fresher platelets (≤ 3 days of storage) can be transfused [7].

Premedication

Premedication of the patient with an antipyretic drug, paracetamol in the UK and acetaminophen in North America, has become standard practice to reduce the risk of FNHTRs. Aspirin should not be used as a premedication for platelet transfusions as it affects platelet function. Some centres premedicate all patients prior to transfusion. However, there are no clinical data to justify this universal approach, except for patients with recurrent FNHTRs, who can be treated with an antipyretic approximately 30 minutes prior to transfusion [17–20].

Allergic Transfusion Reactions

Clinical Presentation

- Allergic transfusion reactions can be either non-systemic/localised or systemic/generalised and are classified as mild, moderate or severe.
- Non-systemic reactions are usually mild, consisting of urticaria and occasionally focal angioedema. These are benign and self-limiting, though they can still cause symptoms that are distressing to the patient. However, such mild reactions may progress to generalised urticaria followed by more severe and systemic reactions with repeated transfusions.
- Systemic reactions range from moderate to severe and life-threatening. Although urticaria is considered a pathognomonic finding for a mild allergic reaction, cutaneous signs or symptoms may be present in severe allergic reactions. Severe reactions usually present with a combination of skin, respiratory or circulatory changes and less commonly with gastrointestinal symptoms. However, approximately 14% of severe reactions present only with respiratory symptoms or hypotension [21].
- Anaphylactic and anaphylactoid reactions behave identically clinically and are managed the same way. These reactions are a medical emergency, as failure to initiate prompt treatment can have fatal consequences. Anaphylaxis usually begins 1–45 minutes after starting the transfusion, but can occur any time during the transfusion and, in addition to an urticarial rash, presents with hypotension/shock, upper or lower airway obstruction (hoarseness, wheezing, chest pain, stridor, dyspnoea, anxiety, feeling of impending doom), gastrointestinal symptoms and, rarely, death.

Differential Diagnosis

To ensure prompt treatment, patients presenting with systemic symptoms should also be quickly evaluated for the following:

- Other causes of respiratory distress, including circulatory overload, TRALI or any other co-morbid conditions such as pulmonary embolism and exacerbations of chronic lung disease.
- Other causes of shock such as acute haemolytic transfusion reactions, sepsis and other co-morbid clinical conditions that can be associated with shock.
- Hypotension with or without cutaneous flushing due to bradykinin (BK) or des-Arg9-BK generation with the use of negatively charged bedside leucocyte-reduction filters, or their accumulation in platelets during storage. Such hypotensive reactions may occur patients being treated with an angiotensin-converting enzyme (ACE) inhibitor or who have inherited a decreased ability to metabolise BK or des-Arg9-BK [22]. These reactions have largely disappeared as bedside leucocyte-reduction filters are now rarely used.

Frequency

It is estimated that about 1% of transfusions are complicated by allergic reactions and that allergic reactions comprise 13–33% of all transfusion reactions. Rates of allergic transfusion reactions vary widely between the studies, depending on product type and preparation. In a review of the studies done between 1990 and 2005 [17]:

- Allergic reactions associated with packed red cell transfusions were reported to range from 0.03% to 0.61%, with a median of 0.15% (1 reaction per 667 transfusions).
- Allergic reactions associated with platelet transfusions occurred at a higher rate, ranging from 0.09% to 21% with a median of 3.7% (1 reaction per 27 transfusions), with more recent studies showing decreased rates associated with the use of PAS [9, 23–25].
- The frequency of allergic reactions associated with the transfusion of plasma was lower than platelets, but more common than reactions to red cells. More recent studies suggest lowered allergic reactions with selected plasma products such as solvent detergent-treated plasma [26].

True anaphylaxis is a systemic reaction caused by antigen-specific cross-linking of immunoglobulin (Ig)E molecules on the surface of tissue mast cells and peripheral blood basophils, with immediate release of potent mediators. In contrast, immediate systemic reactions that mimic anaphylaxis but are not caused by an IgE-mediated immune response are termed anaphylactoid reactions. Both anaphylactic and anaphylactoid reactions are severe and life-threatening, but fortunately they are rare and comprise only about 1.3% of all transfusion reactions, affecting 1/20 000 to 1/47 000 transfusions.

Pathogenesis

Generally, an allergic transfusion reaction is defined as a type I hypersensitivity response mediated by IgE antibodies binding to a soluble allergen and resulting in the activation of mast cells. In these reactions, the allergen is often not known. In contrast, severe reactions such as anaphylaxis that involve IgG anti-IgA are classified as type III reactions. These reactions result in complement activation, with subsequent amplified release of anaphylatoxins C3a and C5a, leading to anaphylaxis.

When the aetiology of an allergic reaction is identified, it usually falls into one of the following categories:

- A recipient with preexisting antibodies to foreign plasma proteins in the blood component.
- Recipient antibodies against a substance in the blood product that either is lacking or has a distinctly different allelic expression in the patient (i.e. IgA, haptoglobin, C4).
- Extraneous substances in the component (i.e. passively transmitted donor IgE antibodies, drugs, other allergens).

For most patients, the underlying aetiology is believed to be a recipient preexisting antibody to plasma proteins in the blood component that cannot be specifically identified. Although deficiencies in IgA, haptoglobin or C4 are usually sought, they are rarely encountered and represent a very small minority of allergic reactions. In some instances, an allergic reaction can be traced back to donor-specific factors, which confers at most a 5% chance of causing an allergic reaction in another recipient; therefore, patient-specific factors are a predominant cause of allergic reactions [27]. Patients with hay fever and food allergies tended to have more allergic reactions, and the severity of reactions is milder in older patients [28].

Management

When any transfusion reaction is suspected, the transfusion is stopped immediately while the patient is clinically assessed. As the signs and symptoms of a transfusion-related allergic reaction are identical to an allergic reaction caused by other allergens, excluding other causes of the reaction, such as concomitantly administered medications, is essential.

- Mild non-systemic allergic transfusion reactions (localised skin reaction or itching) are usually treated with an antihistamine, commonly diphenhydramine 25–50 mg intramuscularly (IM) or intravenously (IV) in North America and chlorphenamine (Piriton®) 10–20 mg IM or IV in the UK. The transfusion can be restarted at a slower rate once symptoms have settled.
- Moderate reactions can also be treated with a dose of corticosteroids and the transfusion of that unit is usually discontinued indefinitely.
- In severe reactions, the unit is never restarted. Anaphylaxis is treated as for any other anaphylactic reaction.
- In addition to discontinuing the current transfusion, other blood components collected simultaneously from the same donor should be identified and avoided for this patient, particularly apheresis platelets, where two or three doses may originate from a single collection. However, the likelihood of donor-specific factors triggering an allergic reaction in a different recipient is low relative to patient-specific factors, so it is considered safe to use other blood components from this donor for other patients [27].

The management strategy for severe allergic reactions differs for adults and paediatric patients. For adults/adolescents, immediate administration of adrenaline (epinephrine in the USA) 500 µg (0.5 mL of 1 : 1000 solution) IM is key. Aggressive volume expansion with IV normal saline, oxygen supplementation and antihistamines is also required. If the hypotension is intractable, adrenaline 500 µg (5 mL 1 : 10 000 solution) IV can be given every 5–10 minutes and preparations should be made to transfer the patient to an ICU where an IV drip of inotropic therapy can be maintained. Intubation may be necessary if the airway becomes compromised.

For paediatric patients, the treatment of anaphylaxis should include adrenaline 10 µg/kg 1 : 1000 concentration IM (e.g. under 6 months: 100 µg or 0.10 mL of adrenaline 1 : 1000; 6 months–6 years: 150 µg or 0.15 mL; 6–12 years, 300 µg or 0.3 mL) that can be repeated every five minutes (maximum dose 500 µg). A µg/kg dose should be used rather than a mL/kg dose, as there are different concentrations of adrenaline. Administration of chlorphenamine (250 µg/kg IV for children 1 month to less than 1 year of age; 2.5–5 mg for 1–5 years; 5–10 mg for 6–12 years; 10 mg for over 12 years) or diphenhydramine 1 mg/kg IV/IM in the USA and ranitidine 1 mg/kg IV (maximum dose 50 mg) are also effective for supportive management.

While these drugs are being prepared, the focus should be on resuscitation, including oxygen therapy, suctioning and positioning of the patient to open the airway, maintenance of the circulation, oxygen saturation monitoring, and administering a fluid bolus with 20 mL/kg sodium chloride 0.9% if venous access is established. If signs and

symptoms persist despite a single IM dose of adrenaline, then an intensive care specialist should be consulted to provide airway and further haemodynamic support.

Prevention

Premedication with Antipyretics and/or Antihistamines

Reportedly 50–80% of transfusion recipients in Canada and the USA are premedicated. However, two systematic reviews of the literature assessing the efficacy of premedication in FNHTRs, including the results of prospective randomised controlled trials (RCTs), found no evidence that premedication prevented allergic or febrile non-haemolytic transfusion reactions [19,20]. A retrospective review of 7900 transfusions in 385 paediatric oncology patients also found no statistically significant difference in allergic transfusion reactions between those who received premedication and those who did not, and no difference in allergic reactions with or without premedication even in those with a previous history of two or more allergic reactions [29]. In addition, allergic reactions were not more common in those with a history of two or more allergic transfusion reactions. Although premedication does not appear to affect the incidence of allergic reactions, there have been no studies to date evaluating whether premedication influences the severity of such reactions. A recent case report suggests possible benefit of off-label use of anti-IgE monoclonal antibodies for premedication in patients with consistently severe allergic reactions [30].

Leucocyte Reduction

Unlike FNHTRs, there is no significant reduction in allergic transfusion reactions with the use of leucocyte-reduced blood products.

Washed Components/Plasma-Reduced Components

Red cells have minimal volumes of residual plasma and would require washing to further reduce the amount of plasma proteins transfused. Washing was associated with a decrease in allergic reaction rates from 2.7% to 0.3% for red cells in one study [31]. Other data suggest minimum benefit with washing of red cells based on various haemovigilance data reports (reviewed in [32]). Furthermore, washing of red cells may lead to other complications associated with increased red cell fragility, haemolysis and hyperkalaemia in the blood product [33,34]. For apheresis platelets collected and stored in PAS, where only 35% of the volume of the product contains plasma, its use was associated with a lowering of allergic reactions from 1.85% to 1.01%, compared to conventional apheresis platelets with 100% of the volume containing plasma [35]. A slight decrease in platelet count increment immediately after transfusion was noted with platelets in additive solution, but no difference in platelet count increment was seen at 12–24 hours [35]. Where manual removal of plasma was attempted with more conventional apheresis platelets in older studies, plasma reduction was associated with a lowering of allergic reactions from 5.5% to 1.7%, and was further reduced with washing to 0.5% [31]. Platelet activation was significantly higher with washing (24.2% increase) than with plasma reduction (10.3% increase). Platelet recovery was better with plasma reduction (80.7%) than with washing (70.5%), which was not considered a significant difference. In contrast, plasma reduction only removed 51.1% of plasma proteins versus 96% with washing [36]. With the exclusion of severe or life-threatening allergic reactions that would benefit from washing of cellular products, the use of plasma reduction was sufficient to decrease the number of allergic reactions in 67.4% of patients with clinically significant or multiple urticarial reactions.

As already mentioned, the growing availability and use of apheresis platelets suspended in PAS with a consequent reduction in plasma have been associated with an overall reduction in the frequency of allergic

transfusion reactions and likely reduce the need for manual plasma washing or plasma reduction.

Immunoglobulin A–Deficient Blood Components

IgA deficiency is the most common primary immunodeficiency in the Western world, affecting up to 1 in 20 people. Severe IgA deficiency, defined as IgA < 0.05 mg/L, can be associated with anaphylactic reactions to blood components, which almost always contain IgA [22]. Patients with anaphylactic transfusion reactions should have further testing using a pretransfusion serum sample to quantify their serum IgA level as well as anti-IgA antibody titres, although few patients with anaphylactic transfusion reactions are IgA deficient. Furthermore, only a small proportion of IgA-deficient recipients have anti-IgA antibodies, of which only a few cases have been documented to have anaphylactic reactions with non-IgA-deficient products [37,38]. Despite the frequency of IgA deficiency in the population, the number of fatal or severe anaphylactic reactions that have been attributable to IgA deficiency has been rare, even raising the question of whether IgA deficiency–related anaphylaxis has a sound evidence base [39].

Despite the weak evidence in the literature, the dilemma remains as to whether IgA-containing blood products are safe for a patient who is suspected of having IgA deficiency or a patient who has had an anaphylactic reaction where anti-IgA has not been determined as the cause. Given the near-ubiquitous presence of IgA in blood products, a patient with a transfusion within the past 24 hours with no reaction is unlikely to have IgA deficiency as their underlying cause of anaphylaxis if it occurs on a subsequent transfusion. If serum IgA is detectable in the patient, anaphylaxis due to IgA deficiency is very unlikely, though not entirely excluded, with 0.7% of patients with low or normal IgA levels having detectable anti-IgA [37]. Even among patients with anti-IgA, only rare cases of associated anaphylaxis were noted [39].

Due to the limited sensitivity of the IgA assay in most hospital laboratories (0.20–50 mg/dL with nephelometry or turbidimetry), additional testing is usually required to identify patients with severe IgA deficiency (less than 0.05 mg/dL) and to test for anti-IgA antibodies in reference laboratories. Because of the delay in confirmatory testing, often requests for additional transfusions are made prior to the availability of results. Given the rarity of this type of transfusion reaction, even among patients with known IgA deficiency, withholding transfusions pending the outcome of laboratory testing or the availability of IgA-deficient or washed blood product may cause greater harm than a slower transfusion with careful monitoring, depending on the clinical urgency for transfusion support [38].

If an allergic transfusion reaction secondary to IgA antibodies due to IgA deficiency is confirmed, IgA-deficient or washed products should be given for any future transfusions.

Even in such circumstances, when faced with a life-threatening need for transfusion prior to the availability of IgA-deficient products, a slow transfusion with intense monitoring and immediate access to supportive care in the event of a severe reaction may outweigh the risk of anaphylaxis, as recurrence of anaphylaxis due to IgA is not a certainty [39]. Such an approach should be taken with extreme caution and only after a discussion about the risks and benefits of administering an unwashed product to such an individual between the transfusion service and the patient's attending physician. Since anaphylactic transfusion reactions are rare and often not due to IgA deficiency, while transfusions are common and often urgent, it is both impractical and not cost-effective to widely screen for IgA deficiency in the pretransfused population.

KEY POINTS

1) Allergic and febrile non-haemolytic transfusion reactions (FNHTRs) are the most common transfusion reactions. Anaphylaxis is rare.
2) Mild allergic reactions usually only require antihistamine treatment, and the transfusion can be continued unless systemic symptoms develop.
3) Mild FNHTRs typically respond to the administration of an antipyretic.
4) If a moderate to severe transfusion reaction is suspected, the transfusion must be stopped until the patient is assessed, and possible causes of the reaction are investigated.
5) Systemic symptoms warrant prompt clinical assessment, as treatment can vary widely between diagnoses and, in particular, failure to administer adrenaline (epinephrine) in anaphylactic reactions can be fatal.
6) As the signs and symptoms of FNHTR and allergic reactions can be mimicked by a variety of other causes, the patient's underlying disease state and any other co-administered medications should be considered in the differential diagnosis. As there is usually no definitive test to determine the aetiology of these reactions, they are generally diagnosed by exclusion.

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10 Transfusion-Associated Circulatory Overload

Robert B. Klanderman[1,2] and Alexander P.J. Vlaar[1]

[1] *Department of Intensive Care, Amsterdam UMC–AMC, University of Amsterdam, Amsterdam, The Netherlands*
[2] *Department of Anesthesiology, Amsterdam UMC–AMC, University of Amsterdam, Amsterdam, The Netherlands*

Transfusion-associated circulatory overload (TACO) is almost as old as transfusion itself. Cases were published as early as 1941, since blood could be stored outside the body and later transfused. A report of five cases describes dyspnoea occurring rapidly following transfusion, eventually resulting in four deaths [1]. Patients suffered 'cardiac embarrassment' with evidence of pulmonary oedema, which today is classified as TACO.

Definition

TACO is defined according to a set of clinical criteria and is a bedside diagnosis (Table 10.1). This combination of symptoms most accurately identifies patients that transfusion experts recognise as having TACO [2]. It is characterised by new or worsening respiratory symptoms or pulmonary oedema following transfusion in combination with haemodynamic signs, biomarkers or evidence of circulatory overload. The revised 2018 TACO definition has increased the onset time of symptoms from 6 to within 12 hours following transfusion.

Incidence and Outcomes

TACO is the most common cause of transfusion-related major morbidity and mortality, surpassing transfusion-related acute lung injury (TRALI) by 2010 according to UK haemovigilance data. TACO is greatly underdiagnosed and underreported [3], likely because dyspnoea is subjective and not always recognised. Active surveillance shows a 1% incidence in patients transfused, with approximately 15% of reported cases occurring after only one unit transfused. In the perioperative and intensive care unit (ICU) settings, the incidence increases to 5.5% of those transfused [4]. The highest incidence of 13% is reported in patients who received multiple plasma transfusions for rapid reversal of vitamin K antagonists [5]. Use of convalescent plasma during the COVID-19 pandemic showed a 1% incidence of TACO in patients with moderate or severe COVID [6].

TACO is associated with an increased hospital length of stay (median 4.5 days), ICU admission and need for mechanical ventilation. Critically ill patients who develop TACO while in ICU also have a longer length of stay (median 1.5 days). Mortality is approximately

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Table 10.1 Revised 2018 diagnostic surveillance criteria for transfusion-associated circulatory overload (TACO).

Required criteria (at least one)	
A) Acute or worsening respiratory compromise	
B) Evidence of acute or worsening pulmonary oedema	Based on (and/or): • Clinical physical examination • Radiographic chest imaging • Other non-invasive assessment of cardiac function
Additional criteria	
C) Cardiovascular system changes (not explained by patient's underlying medical condition)	Including (and/or): • Tachycardia • Hypertension • Jugular venous distention • Enlarged cardiac silhouette • Peripheral oedema
D) Evidence of fluid overload	Including (and/or): • Positive fluid balance • Clinical improvement following diuresis
E) Supportive result of a relevant biomarker	B-type natriuretic peptide levels (BNP or NT-proBNP): • Above age group–specific reference range and > 1.5 times the pretransfusion value
Patients should exhibit at least one 'required' criterion during or up to 12 hours after transfusion and a total of ≥ 3 out of 5 criteria (A to E).	

Source: Adapted from Wiersum-Osselton et al. [2].

10% [7,8] and long-term survival at one and two years' follow-up remains decreased, although this was not significant [9].

Clinical Manifestations

TACO is hallmarked by respiratory distress with evidence of volume overload; however, the time course and severity of symptoms can differ widely. Symptoms can develop rapidly (within minutes) after initiating the transfusion or have a more insidious onset (up to 12 hours). Dyspnoea is the most common presenting symptom. Alternatively, patients might not experience dyspnoea, but increased breathing frequency or greater respiratory effort may be observed. Patients will prefer a seated position and become increasingly dyspnoeic when supine. Haemodynamic changes include tachycardia and specifically hypertension. Employing pulse oximetry and monitoring vital signs can help identify cases as they occur. Fever has also been associated with TACO, occurring in up to a third of cases.

An ABCDE approach should be used, as rapid recognition and treatment of respiratory distress are paramount. A physical examination should include looking for cyanosis, any respiratory distress, the respiratory frequency and oxygen requirements. A lung examination can reveal lung crackling or rales, likely in the lower or dependent lung fields. Pitting oedema of the lower extremities (or sacrum in supine patients) may be present as sign of preexisting volume overload, renal insufficiency or heart failure. Very severe cases of TACO may present with symptoms of decompensated cardiac shock, including cold extremities, decreased capillary refilling, oliguria and altered mental status.

Dyspnoea can rapidly progress to type I respiratory failure as pulmonary oedema accumulates. Patients will have rapid shallow breathing and profound hypoxaemia despite supplemental oxygen. Alternatively, type II respiratory failure due to exhaustion can occur over a longer period of time, characterised by a rising partial pressure of carbon dioxide ($PaCO_2$). Patients already mechanically ventilated who develop TACO, i.e. in the operating room (OR) or ICU, may show a decrease in pulmonary compliance due to pulmonary oedema and increased oxygen requirements.

Pathophysiology

Development of TACO is through an iatrogenic rapid increase in circulating volume, a situation humans have evolutionarily not needed to compensate for. Discussion is ongoing as to how blood transfusion resulting in circulatory overload differs from conventional crystalloid overload. There is increasing evidence that when giving an equivalent volume of blood or fluid, transfusion will sooner result in circulatory overload [10]. Furthermore, a number of studies propose a higher incidence of TACO occurring after plasma transfusion, suggesting circulatory overload is not solely volume dependent.

The underlying pathological mechanisms are a topic of ongoing research, but the final common pathway is the same. Transfused volume overloads the heart, thereby left-ventricular (LV) end-diastolic volume (Figure 10.1) and pressure increase, resulting in hydrostatic pressure in the pulmonary capillaries. According to Starling's forces, hydrostatic pressure forces transudate through the thin capillary wall into the alveoli [11]. The lung is likely to be the first organ to become symptomatic since the alveolar membrane is very thin, to facilitate diffusion, and the alveoli filled with air provide no resistance to hydrostatic pressure. Gas exchange in the alveoli is impeded by pulmonary oedema, leading to respiratory distress, dyspnoea and hypoxaemia.

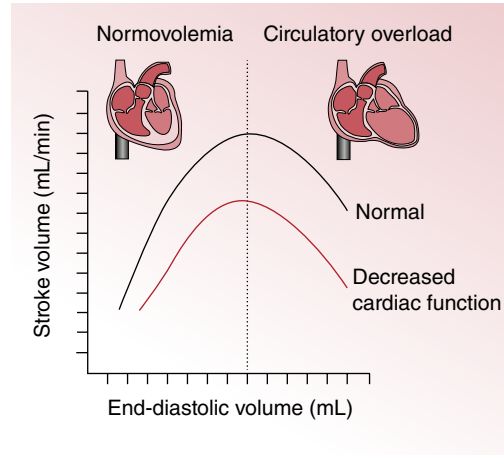

Figure 10.1 The Frank–Starling curve showing the relationship between the contractility of the myocardium and the filling of the heart. When the heart is volume overloaded the myocardium stretches beyond the zenith and the left ventricle is unable to effectively contract. Blood backs up into the left atrium and pulmonary vasculature, increasing hydrostatic pressure, which leads to pulmonary oedema.

The haemodynamic effects accompanying TACO include hypertension and tachycardia. The physiological explanation for this is potentially due to sympathetic activation as a result of forward failure. The decrease in cardiac output, through baroreceptor reflexes, causes sympathetic activation, resulting in vasoconstriction and hypertension as well as tachycardia [12].

Risk Factors

Primary risk factors for developing TACO include the extremes of age, a positive fluid balance, preexisting renal disease and myocardial dysfunction, number of units transfused and speed of transfusion (Table 10.2). These factors mostly have in common that they interfere with or overwhelm haemodynamic compensatory mechanisms to accommodate a sudden increase in circulating volume.

Extremes of Age

Young children are prone to develop TACO, though the reason is still unclear. In the

Table 10.2 Risk factors for transfusion-associated circulatory overload (TACO).

Risk factor	Author	Study type	Metric	Outcome
Age	Thalji 2018	Active retrospective	Age ≤ 8 yrs vs > 8 yrs	≤ 8 yrs: 5.2%
				> 8 yrs: 1.0%
	Menis 2011	Passive retrospective	Age 65–69	OR 1.0 (ref)
			Age 70–74	OR 1.3 (1.2–1.5)
			Age 75–79	OR 1.3 (1.1–1.5)
			Age 80–84	OR 1.7 (1.4–2.0)
			Age ≥ 85	OR 2.1 (1.7–2.5)
Cardiac dysfunction	Refaai 2015	Prospective	History of heart failure	OR 2.4 (1.1–5.2)
	Murphy 2013	Active retrospective	""	OR 6.6 (2.1–21.0)
	Bosboom 2018	Active retrospective	""	OR 2.4 (1.2–4.6)
	Roubinian 2018	Active retrospective	""	OR 2.0 (1.2–3.5)
	Menis 2011	Passive retrospective	""	OR 1.6 (1.4–1.9)
	Clifford 2017	Active retrospective	Left ventricle dysfunction	OR 1.8 (1.1–3.1)
Renal dysfunction	Refaai 2015	Prospective	Chronic kidney disease	OR 6.6 (2.1–21.0)
	Clifford 2017	Active retrospective	""	OR 2.1 (1.2–3.7)
	Roubinian 2018	Active retrospective	Acute kidney injury	OR 1.9 (1.1–3.1)
	Roubinian 2018	Active retrospective	Renal replacement therapy	OR 3.3 (1.3–8.4)
	Bosboom 2018	Active retrospective	""	OR 2.2 (1.1–4.3)
Number of units transfused	Li 2011	Active retrospective	Number of units	OR 1.5 (1.1–1.9)
	Murphy 2013	Active retrospective	""	OR 1.1 (1.0–1.2)
Transfusion speed	Li 2011	Active retrospective	Transfusion rate	OR 1.9 (1.1–3.3)
Positive fluid balance	Murphy 2011	Active retrospective	Positive fluid balance	OR 9.4 (3.1–28.0)
	Roubinian 2018	Active retrospective	""	OR 1.5 (1.3–1.7)
	Li 2011	Active retrospective	""	OR 1.4 (1.1–1.7)

OR, odds ratio; ref, reference group.
Source: Adapted from Bosboom et al. 2019 [4].

elderly numerous factors can limit haemodynamic accommodation to rapid volume expansion, including but not limited to atherosclerosis and a decreased vascular elasticity, blunting of baroreflexes and physiological decline of renal function. Moreover, with increasing age the likelihood of having one or more additional co-morbidities increases.

Fluid Balance

The baseline volume status is an important factor in TACO, and fluid balance or change in body weight is used as a surrogate marker. While there is little evidence to support this, logically hypovolaemic patients with an intravascular volume deficit can accommodate a volume increase. At increased risk of TACO are euvolaemic or even hypervolaemic patients, e.g. patients admitted to hospital receiving liberal maintenance fluid or with co-morbid heart or renal failure.

Renal and Cardiac Dysfunction

Renal and cardiac dysfunction are known major risk factors for TACO. Chronic kidney disease is characterised by peripheral oedema

and chronic hypervolaemia. The ability to increase diuresis in the setting of volume expansion can be impaired. The renin–angiotensin–aldosterone system (RAAS) is chronically upregulated and atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) will have less effect on a damaged kidney. Hypervolaemia, limited diuresis and hormonal disruption affect haemodynamic compensatory mechanisms to accommodate transfused volume.

Myocardial dysfunction limits the heart's ability to accommodate increased preloads. In patients with a low ejection fraction (< 40%), circulatory overload results from overextension of a weakened LV past the apex of the Frank–Starling curve (Figure 10.1). A vicious circle ensues when the LV dilates, blood accumulates and the LV is further dilated. Backward failure leads to increases in left-atrial pressures and hydrostatic pulmonary capillary pressure rises. In heart failure with preserved ejection fraction, diastolic dysfunction results in backward failure. Here the non-compliant, or stiff, ventricle is suddenly challenged with an increased preload and it cannot dilate to accommodate larger volumes. Blood accumulates in the left atrium and backward failure again leads to TACO.

Transfusion Volume and Rate

Transfused volume is not often a modifiable risk factor in a targeted transfusion strategy. Successful steps to reduce the number of transfusions include a 'single-unit' transfusion policy and patient blood management programmes. The rate of transfusion has also been shown to increase hydrostatic pulmonary capillary pressure in transfused patients with a pulmonary artery catheter [13]. In non-emergency settings the rate of transfusion is a modifiable risk factor. Specifically for patients with risk factors for TACO, a prudent estimation of a patient's ability to tolerate transfused volume should be undertaken and the transfusion rate adjusted accordingly.

Type of Product

Retrospective studies show that plasma transfusions most increase the odds of developing TACO, followed by red blood cells and then platelets. While indication bias may cloud the findings, mechanistically different blood product constitutions could activate different pathological mechanisms; this topic is still under investigation [4].

Differential Diagnosis and Evaluation

The differential diagnosis is dependent on the presenting symptom, time of onset, progression of symptoms as well as their severity. Respiratory distress can manifest as dyspnoea, laboured breathing, tachypnoea and/or hypoxemia. The differential should include TACO, TRALI, transfusion-associated dyspnoea (TAD), anaphylactic transfusion reaction, febrile non-haemolytic transfusion reaction (FNHTR), ABO incompatibility and even transfusion-related bacterial sepsis. Differentiating TACO from TRALI is likely to be the most challenging. However, there are a number of characteristics that can help in this (Table 10.3).

Patient Characteristics and Onset of Symptoms

Based on patient characteristics and co-morbidities, a high-risk population can be defined in which TACO is more likely to occur (discussed under the TACO risk factors heading). Less characteristic is TACO in healthy adults without co-morbidities receiving few transfused units [14]. The onset of symptom can be earlier in TRALI, with symptoms in a healthy volunteer occurring after only 50 mL of blood is transfused. Furthermore, diagnostic criteria dictate that TRALI should be apparent within 6 hours and TACO within 12 hours after completing a transfusion [2].

Table 10.3 Differentiating transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI).

Symptoms		TACO	TRALI
Pulmonary	Respiratory worsening/distress • Dyspnoea • Tachypnoea • Hypoxemia	Present	Present
Vital signs	Heart rate	Tachycardia	Tachycardia
	Blood pressure	Hypertension	Hypotension
	Temperature	Normal (febrile in 1/3 cases)	Febrile
	Oxygen saturation	Hypoxaemia	Hypoxaemia
Risk factors	Cardiac dysfunction: • History of heart failure • LV dysfunction	Increased risk	Non-contributory
	Renal dysfunction: • Chronic kidney disease • Acute kidney injury	Increased risk	Non-contributory
	Fluid balance	Positive	Non-contributory
Physical exam	Cyanosis	Possible	Possible
	Heart examination • Cardiac apex • Auscultation	 Displaced S3 gallop	 Normal Normal
	Lung auscultation	Rales/crackles more in dependent lung fields	Rales/crackles throughout
	Extremities	Peripheral pitting oedema	No peripheral oedema
Laboratory evaluation	Arterial blood gas	Hypoxaemia	Hypoxaemia
	WBC count	Normal	Decreased
	Cardiac biomarkers: • BNP (NT-proBNP) • Troponin	Elevated	Normal (may become elevated in severe disease)
Radiological evaluation	Chest X-ray/chest CT: • Pulmonary oedema • Vascular pedicle width • Chest–thorax ratio	 Bilateral in dependent fields. > 65 mm > 0.55	 Bilateral throughout < 65 mm < 0.55
	Lung ultrasound: • 'A lines' • 'B lines'	 Absent > 3 in dependent lung fields	 Absent > 3 throughout the lungs
Echocardiography	Left-atrial volume	Increased	Normal/decreased
	Ejection fraction	Decreased (< 40%)	Normal (> 40%)
	Diastolic dysfunction	Present	Absent
Invasive measurements	Central venous pressure	Elevated	Normal/decreased
	Pulmonary artery catheter*	Elevated (PCWP > 18)	Normal/decreased
	Bronchoalveolar lavage*	Transudate	Exudate

Findings that when present can aid in differentiating between TACO and TRALI. Not all signs need be present for the diagnosis and the absence of symptoms does not rule out the diagnosis.
*Pathognomonic signs/symptoms for, respectively, TACO and TRALI.
BNP, B-type natriuretic peptide; CT, computed tomography; LV, left ventricle; NT-proBNP, N-terminal proBNP; PCWP, pulmonary capillary wedge pressure; WBC, white blood cell.

Symptoms

Respiratory symptoms cannot differentiate between TACO and TRALI. Anaphylactic transfusion reactions can present with respiratory symptoms, namely bronchospasm, but rarely present with pulmonary oedema. Hypertension is more characteristic of TACO through sympathetic activation, as opposed to TRALI in which vascular permeability leads to hypovolaemia and hypotension. Fever is common in TRALI; however, up to a third of TACO cases are also associated with fever [15]. Bacterial contamination, FNHTR and a haemolytic transfusion reaction can also present rapidly after initiating a transfusion. Symptoms include tachypnoea, fever, chills and, in the case of both bacterial contamination and haemolytic transfusion reactions, also hypotension. However, these are not pulmonary transfusion reactions and both pulmonary examination and pulse oximetry should be normal. Respiratory symptoms in the absence of pulmonary oedema, or those that do not meet diagnostic criteria of either TACO or TRALI, point to TAD.

Laboratory Assessment

Diagnostic workup includes an arterial blood gas analysis to ascertain the degree of hypoxaemia and the pulmonary oxygenation capacity (PaO_2–FiO_2 [fraction of inspired oxygen] ratio), but this will not differentiate TACO from TRALI. Specific to TRALI is a decreased white blood cell count due to immune activation. Elevated cardiac stress biomarkers including BNP and NT-proBNP (a cleavage fragment of the BNP prohormone) are associated with TACO. A systematic review concluded that patients were unlikely to have TACO with a BNP below 300 pg/mL or NT-proBNP below 2000 pg/mL [16]. Severe cases of TACO and TRALI can have elevated BNPs, but the studies to date have been unable to differentiate these two based on BNPs alone.

Radiological Evaluation

A chest X-ray (CXR) can be used to show pulmonary oedema, but specificity in the acute setting is only 70%. It is however useful to exclude other lung pathology that can present with dyspnoea. Signs of volume overload include (i) a vascular pedicle width > 65 mm as a sign of pulmonary vascular congestion; (ii) increased chest–thorax ratio > 0.55 indicating either chronic heart disease or acute cardiac dilation; and (iii) Kerley B lines, intralobular septal thickening that is more typical of hydrostatic than of permeability oedema [17]. Cut-off values for vascular pedicle width and chest–thorax ratios are based on standing patients with posterior-anterior (PA) CXRs; cut-off values for supine patients with anterior-posterior (AP) CXRs are not defined [18]. A chest computed tomography (CT) scan can provide slightly more information, but it is more laborious, will require the patient to remain supine and differentiation of TACO and TRALI remains difficult.

Lung ultrasound is a point-of-care method to rapidly detect pulmonary oedema and can also be used to exclude other diagnoses, including pleural effusion, pneumonia or atelectasis. The absence of 'A lines' and the presence of ≥ 3 B lines indicates pulmonary oedema. Since there may be regional differences in TACO, ultrasound also allows comparison of both dependent and non-dependent lung fields.

Echocardiography

Transthoracic echocardiography (TTE) is a minimally invasive tool that can rapidly support or exclude the diagnosis of TACO, although there are no set criteria. Findings suggestive of TACO include those of left-atrial hypertension, which include dilation of the LV and increased left-atrial volume. Secondary signs include a dilated right ventricle (RV) and elevated pulmonary artery pressures measured by ultrasound. Conditions predisposing to TACO include LV hypertrophy and LV dysfunction, i.e. an

ejection fraction < 40% or diastolic dysfunction. TACO becomes unlikely if left-atrial volume is normal or decreased or in case of an empty LV at end systole seen as 'kissing walls'.

Invasive Measurements

Central venous pressure (CVP) measurement requires a central venous catheter. Values above 12 mmHg are elevated and can indicate hypervolaemia; however, this is a non-specific sign that can also indicate RV backward failure. CVP is also a poor discriminator between severe TACO and TRALI, since in both situations pulmonary vascular resistance can increase and right-sided backward failure can occur. A pulmonary artery catheter can be used to measure pulmonary capillary wedge pressure (PCWP), the gold standard for left-atrial pressure estimation. A PCWP of > 18 mmHg in combination with pulmonary oedema will rule out TRALI and confirm the diagnosis of TACO. Finally, determination of bronchoalveolar lavage protein/serum protein ratio will theoretically discern between a transudate, which is fitting for TACO, versus an exudate, which is characteristic for permeability oedema. This is invasive, however, and to date no studies have validated this method.

Treatment and Prevention

Any time a patient becomes dyspnoeic during transfusion and an adverse reaction is suspected, the transfusion should be stopped immediately. In TACO the goals of treatment include first supportive care for respiratory symptoms, and second treatment of circulatory overload. Initial management includes placing the patient upright and administration of supplemental oxygen. Early placement of a urinary catheter and recording an accurate fluid balance will aid in monitoring treatment.

Rapid progression of symptoms should alert clinicians that the patient may soon progress to respiratory failure and early specialist consultation is in order. Positive pressure ventilation can be beneficial, as it decreases the patient's work of breathing and positive pressure in the alveoli directly opposes hydrostatic pulmonary capillary pressure. Non-invasive systems can include high-flow nasal oxygen and continuous positive airway pressure (CPAP), or intubation and mechanical ventilation can be required.

Treatment of circulatory overload is with diuretics. Loop diuretics are thought to induce an acute vasodilatory effect, which decreases hydrostatic pulmonary capillary pressure. Furosemide is most commonly used and acts to reduce afterload, decreasing cardiac work, and as a loop diuretic promoting natriuresis and lowering circulating volume. Furosemide doses should be patient tailored: commonly used dosages are intravenous administration of 20–40 mg in adults. In cases with renal or cardiac dysfunction, larger doses may be required. Relief of symptoms can occur within minutes of administration. Care should be taken in cases presenting with shock, as diuretics may precipitate hypotension. Alternative therapies include nitrates and inotropes to optimise cardiac function; specifically the latter will require specialised care with continuous monitoring. Finally, ultrafiltration methods to decrease circulating volume, such as dialysis or continuous veno-venous hemofiltration, have been suggested [4].

To prevent TACO, recognition of at-risk patients prior to transfusion is important. Elderly patients with one or multiple risk factors will require a more careful transfusion strategy compared to healthy young adults. In the non-emergent setting transfusion speed can be limited to one unit in four to six hours (depending on national guidelines). Blood banks will sometimes be able to aliquot a unit of red blood cells into smaller portions, even enabling a transfusion over days. The first pilot study in humans has investigated the prophylactic use of furosemide to prevent TACO, the results of which have not given an indication of treatment effect yet [19].

Finally, an episode of TACO should be documented in the patient's chart to alert clinicians to a potential risk of TACO during future transfusions. While there is currently no evidence on recurrence rates, without change of the patient's condition or co-morbidities they likely remain at increased risk. Reporting TACO cases to the centre's haemovigilance officer, blood bank or blood supplier is mandatory in many countries, and aids in research efforts to further understand the incidence, patient population and outcomes [20].

KEY POINTS

1) Transfusion-associated circulatory overload (TACO) is a syndrome of respiratory distress caused by pulmonary oedema due to increased hydrostatic pulmonary capillary pressure.
2) Diagnosis is based on clinical symptoms of respiratory distress or signs of pulmonary oedema combined with evidence of haemodynamic changes, circulatory overload or elevated cardiac stress biomarkers.
3) Risk factors include the extremes of age, fluid balance, preexisting cardiac and renal disease, transfusion speed and volume.
4) Differentiating TACO from transfusion-related acute lung injury (TRALI) can be difficult; a combination of differentiating signs and symptoms, imaging, biomarkers and response to treatment might be required to discern between them.
5) Biomarkers including a B-type natriuretic peptide (BNP) < 300 pg/mL and NT-proBNP < 2000 pg/mL make the diagnosis of TACO improbable in non-critically ill patients.
6) Treatment is supportive respiratory care, including upright patient positioning, administration of supplemental oxygen as well as early administration of loop diuretics. Urinary catheterisation and recording fluid balance will facilitate monitoring of treatment.
7) Prophylactic use of furosemide is promising and should be considered in at-risk patients, even though there is no high-grade evidence to support it. Research on this topic is still ongoing.
8) Document suspected cases of TACO in the patient chart to alert clinicians that the patient might be prone to circulatory overload during subsequent transfusion episodes.
9) Report suspected cases to the local haemovigilance officer or blood bank to improve overall awareness and research efforts to better understand TACO.

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11 Transfusion-Related Acute Lung Injury (TRALI)

Alexander P.J. Vlaar[1] *and Rick Kapur*[2]

[1] *Department of Intensive Care, Amsterdam UMC–AMC, University of Amsterdam, Amsterdam, The Netherlands*
[2] *Sanquin Research; Landsteiner Laboratory, Amsterdam UMC-AMC, Amsterdam, The Netherlands*

Definition

The clinical syndrome of transfusion-related acute lung injury (TRALI) is characterised by the acute onset of respiratory distress during or within six hours of transfusion. The first international consensus definition of TRALI was established in 2004 [1]. In the past decades, insights in the pathophysiology of TRALI has led to the need for an updated international TRALI definition. Based on this accumulated clinical and basic science knowledge, a panel of 10 international experts on TRALI, including two members with haemovigilance expertise, used the Delphi approach to develop the recently published redefinition of TRALI [2].

The updated TRALI definition/classification scheme is shown in Table 11.1 [3]. The main modifications are:

- The term 'possible TRALI' has been dropped.
- TRALI has been separated into two types. TRALI type I applies to cases without an acute respiratory distress syndrome (ARDS) risk factor and TRALI type II to cases with an ARDS risk factor (Table 11.1) or with mild preexisting ARDS. Notably, the presence of either an ARDS risk factor or mild ARDS does not exclude the diagnosis of TRALI as it did under the old definition.
- Cases with an ARDS risk factor that meet ARDS diagnostic criteria and where respiratory deterioration over the 12 hours prior to transfusion implicates the risk factor as causative should be classified as ARDS rather than TRALI type II.
- The 2012 updated ARDS consensus definition (referred to as the Berlin ARDS definition) has been evaluated for its relevance to TRALI and essential updates (including updating the list of commonly accepted ARDS risk factors and expanding the methods for diagnosing hydrostatic pulmonary oedema) have been incorporated into the new TRALI definition (Table 11.1).

Incidence, Outcomes and Recipient Risk Factors

Because TRALI is under-recognised and under-reported, the most accurate data on TRALI incidence rates come from research studies that use an active surveillance mechanism to detect cases [3–5]. With this approach, an overall risk of 1 : 5000 transfused units in the general hospital population was reported

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Table 11.1 Updated transfusion-related acute lung injury (TRALI) definition.

TRALI type I – Patients who have no risk factors for ARDS and meet the following criteria:

a. i. Acute onset

ii. Hypoxemia $PaO_2/F_iO_2 \leq 300$* or $SpO_2 < 90\%$ on room air

iii. Clear evidence of bilateral pulmonary oedema on imaging (e.g. chest radiograph, chest CT or ultrasound)

iv. No evidence of LAH† or, if LAH is present, it is judged not to be the main contributor to the hypoxemia

b. Onset during or within 6 hours of transfusion‡

c. No temporal relationship to an alternative risk factor for ARDS

TRALI type II – Patients who have risk factors for ARDS (but who have not been diagnosed with ARDS) or who have preexisting mild ARDS (PaO_2/F_iO_2 of 200–300), but whose respiratory status deteriorates§ and is judged to be due to transfusion based on:

a. Findings as described in categories a and b of TRALI type I, and

b. Stable respiratory status in the 12 hours prior to transfusion

* If altitude is higher than 1000 m, the correction factor should be calculated as follows: [(PaO_2/FiO_2) × (barometric pressure/760)].

† Use objective evaluation when LAH is suspected (either imaging, for example by echocardiography, or invasive measurement such as pulmonary artery catheter).

‡ Onset of pulmonary symptoms (e.g. hypoxemia – lower P/F ratio or SpO_2) should be within 6 hours of end of transfusion. The additional findings needed to diagnose TRALI (pulmonary oedema on a lung imaging study and determination of lack of substantial LAH) would ideally be available at the same time, but could be documented up to 24 hours after TRALI onset.

§ Use PaO_2/F_iO_2 ratio deterioration, other respiratory parameters and clinical judgement to determine progression from mild to moderate or severe ARDS.

ARDS, acute respiratory distress syndrome; CT, computed tomography; F_iO_2, fraction of inspired oxygen; LAH, left-atrial hypertension; PaO_2, partial pressure of oxygen; SpO_2, oxygen saturation.

Source: Adapted from Vlaar et al. 2019 [2].

in 1985 and this incidence number is frequently cited in the literature [3]. However, more recent prospective studies in high risk patient populations such as the critically ill report incidences of up to 15% of patients transfused being. These studies underline the underdiagnosing and underreporting as well as that critically ill patients are at increased risk for TRALI compared to the general patient population [6-8]. A recent case–control study, which used computer-generated automatic alerts to detect respiratory distress after transfusion, reported a TRALI incidence of 1 : 12 000 units following risk-mitigation procedures for transfused plasma components [5].

Haemovigilance (HV) data are informative about the clinical significance of TRALI. In the USA, TRALI remains a significant cause of transfusion-associated fatalities reported to the Food and Drug Administration (FDA) [9].

Pulmonary infiltrates are thought to resolve in the majority of TRALI patients within 96 hours. Mortality in the general patient population is approximately 10–15%. Those who recover generally do not have any chronic sequelae. In the critically ill patient TRALI has a significant impact on outcome: mortality has been reported to be up to 40% [10].

Most cases occur in adults, but children also acquire TRALI at similar rates [11]. Previous transfusion history is unremarkable and recurrent TRALI is extremely rare. At least one case of TRALI from an autologous transfusion has been recorded. Directed donations from mother to child can cause TRALI due to maternal leucocyte antibodies against the child's leucocyte antigens.

Risk factors related to co-existing recipient medical conditions have been identified by

Table 11.2 Conditions historically associated with transfusion-related acute lung injury (TRALI) and acute respiratory distress syndrome (ARDS).

I. Conditions historically associated with TRALI and pTRALI that are also major ARDS risk factors in the Berlin definition*

- Sepsis†
- Non-cardiogenic shock‡
- Massive transfusion§

II. Conditions historically associated with TRALI (or both TRALI and ARDS) but not listed as major ARDS risk factors in the Berlin definition*

- Cardiac surgery
- Increased pretransfusion plasma IL-8
- Mechanical ventilation with peak airway pressure > 30 cmH_2O
- Chronic alcohol abuse
- Current smoker
- Positive fluid balance
- Higher APACHE II score
- Increased age
- End-stage liver disease
- Postpartum haemorrhage
- Liver transplantation surgery
- Thrombotic microangiopathy
- Surgery requiring multiple transfusions
- Haematological malignancy

* Of note is that patient conditions are variably associated with TRALI in published series due to differences in study design, including type of patient population, differing case mixes of TRALI and pTRALI prospective versus retrospective, and passive versus active reporting. See [13] for a full discussion of the Berlin definition of ARDS.
† Sepsis: For analysis purposes, several studies combined TRALI and pTRALI cases.
‡ Non-cardiogenic shock: In one study in which non-cardiogenic shock was shown to be a TRALI risk factor, the definition of non-cardiogenic shock excluded cardiogenic and septic shock while including haemorrhagic shock and ICU patients on vasopressors for hypotension associated with sedation.
§ Historically, prior to the 2004 CCC, massive transfusion was associated with ARDS. This finding is partially explained by its strong association with TRALI. Studies performed prior to implementation of plasma TRALI risk-mitigation strategies have shown that massive transfusion may serve as a 'second hit' for antibody-mediated TRALI or as a 'first hit' when the transfusions were given prior to the 6-hour time frame.
NB: The conditions listed in this table are not diagnostic criteria for TRALI types I and II.
CCC, Canadian Consensus Conference; ICU, intensive care unit; IL, interleukin; pTRALI, possible transfusion-related acute lung injury.
Source: Adapted from Vlaar et al. 2019 [2].

several large retrospective and prospective observational cohort studies (Table 11.2) [12].

Clinical Manifestations

In non-intubated patients, the onset of TRALI is often quite dramatic, with symptoms starting either during the transfusion or within two hours of its completion in the majority of cases. The syndrome manifests as acute respiratory distress due to non-cardiogenic (inflammatory) pulmonary oedema, and is characterised by acute onset of dyspnoea, tachypnoea and oxygen desaturation. The patient may appear cyanotic and may develop hypotension. Oxygen desaturation is often severe, requiring mechanical ventilation in 70% of cases. Patients with TRALI may experience a low-grade fever for several hours.

Symptoms and signs may be muted in patients under general anaesthesia or already on mechanical ventilation in the intensive care unit (ICU). In such cases, the first indication of TRALI is a drop in the partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) ratio. In rare cases there is the appearance of copious amounts of pink frothy sputum from the endotracheal tube, although this finding is not specific for TRALI.

Hypoxaemia, defined as PaO_2/FiO_2 < 300 mmHg or oxygen saturation of < 90% on room air by pulse oximetry, and the development of new bilateral lung infiltrates on chest radiography, ultrasound or computed tomography (CT) scan are essential in making a diagnosis of TRALI. Chest radiography may show 'whiteout', a radiographic finding in which both lungs show uniform white opacities throughout (Figure 11.1). More commonly, pulmonary infiltrates are located peripherally, especially in the lower lung fields, although in some cases the X-ray changes may be subtle. However, TRALI may also result in more subtle changes on the X-ray that can easily be missed.

Although some investigators have reported that TRALI can rarely have a delayed onset (> 6 hours post transfusion), HV systems do not currently classify such cases as TRALI.

Differential Diagnosis

An important syndrome in the differential diagnosis of TRALI is transfusion-associated circulatory overload (TACO), which similarly results in hypoxaemic respiratory insufficiency and manifests with pulmonary oedema. However, in contrast to TRALI, the pulmonary oedema in TACO arises due to elevated pulmonary vascular pressures and is therefore hydrostatic in nature rather than inflammatory. In line with its underlying pathophysiology, TACO characteristically arises in the setting of one or more features suggestive of fluid overload or congestive heart failure. Frequently, risk factors for both TRALI and TACO are present and, to further complicate matters, there has been an increased recognition that both TRALI and TACO may co-exist as contributors to posttransfusion pulmonary oedema, further challenging the specific diagnosis underlying posttransfusion hypoxaemic respiratory insufficiency [14].

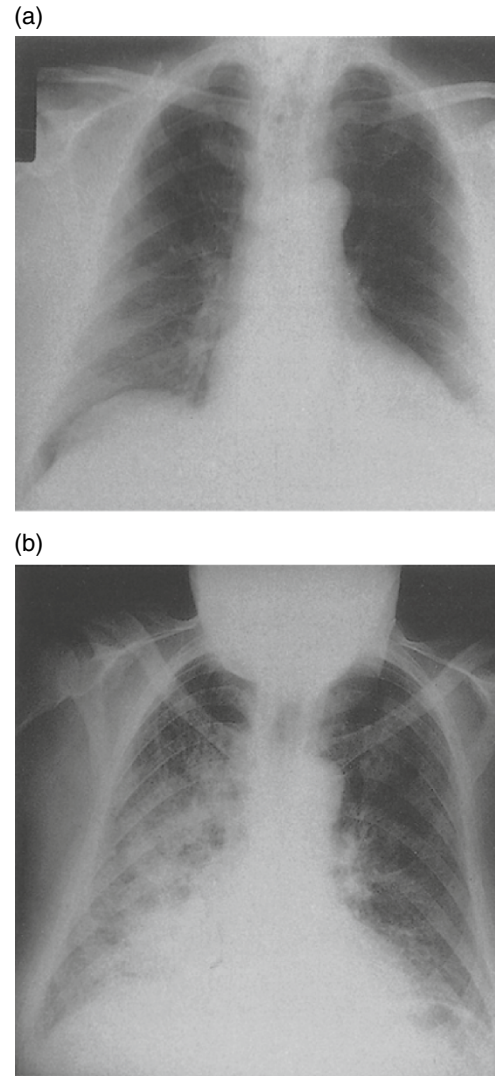


Figure 11.1 Chest X-rays of a patient with transfusion-related acute lung injury. (a) One day before a platelet transfusion and (b) shortly after transfusion showing diffuse bilateral shadowing of the lungs and a normal-sized heart. *Source:* Virchis et al. Lesson of the week: acute noncardiogenic lung oedema after platelet transfusion. *BMJ* 1997;314:880. Reproduced with permission of BMJ Publishing Group Ltd.

There is no single finding that specifically differentiates TRALI from TACO. The lack of certain clinical findings and the presence of others may help in the differential diagnosis (Table 11.3) [5,15]. It has been reported that a 50% elevation of B-type natriuretic peptide (BNP) in a posttransfusion versus a pretransfusion sample supports a TACO diagnosis, while a BNP level of < 200 pg/mL measured immediately after the onset of acute pulmonary oedema supports the diagnosis of TRALI. In critical care patients, BNP or N-terminal-BNP levels may be higher in patients who develop TACO compared to those who develop TRALI; however, there is some concern that these analytes have limited diagnostic value due to a large overlap among the observed values in these patient groups [16,17]. Currently, BNP and NT-proBNP are the primary diagnostic biomarkers researched for TACO. An NT-proBNP ratio greater than 1.5 is supportive of TACO, and low levels of BNP or NT-proBNP can exclude TACO. However, they are unreliable in critically ill patients. Other biomarkers, including cytokines and pulmonary oedema fluid-to-serum protein ratio, have not yet been sufficiently investigated for clinical use [18].

In addition to TACO, other conditions that can mimic TRALI include anaphylactic transfusion reactions and sepsis from transfusion of bacterially contaminated blood components. Bronchospasm, wheezing, localised or generalised skin rash and hypotension or shock favour a diagnosis of anaphylactic reaction. High fever, chills, rigor, shock, disseminated intravascular coagulation, a positive Gram stain and culture from the transfused blood component and positive blood cultures from the recipient support a diagnosis of transfusion-transmitted bacterial sepsis. Finally, a low-grade fever seen in TRALI must be differentiated from a haemolytic transfusion reaction. A clerical check of the transfusion episode showing the lack of any error, plus an absence of visual haemolysis in the posttransfusion serum or plasma and a negative direct antiglobulin test, suggest that a haemolytic transfusion reaction is unlikely.

The term 'transfusion-associated dyspnoea' (TAD) has been endorsed for use in HV systems; it designates cases of posttransfusion dyspnoea that do not fit into any of the known pulmonary transfusion reaction categories. Such cases appear to be non-specific and may be characterised either by less severe pulmonary compromise or by delayed onset falling outside the six-hour interval that defines TRALI; they should not be classified as mild or delayed TRALI as there are no standard definitions for these conditions. (See Table 11.4 for a full classification of pulmonary oedema not fulfilling TRALI criteria.)

Pathogenesis

Two-Hit Model

The pathogenesis of TRALI is complex and incompletely understood. Generally, the pathogenesis can be viewed as a two-hit model, where the first hit represents the underlying clinical condition of the transfused recipient and the second hit is conveyed by components in the transfused blood product [19,20]. Therefore, the first hit is a priming factor, and the additional second hit conveyed by the blood transfusion results in the onset of TRALI. Both hits together, via multiple and yet unresolved immunological mechanisms, result in damage to the pulmonary endothelium resulting in permeability pulmonary oedema, which is responsible for the acute respiratory distress and development of acute lung injury. Figure 11.2 shows a lung section of a fatal TRALI case.

First-hit factors in the transfused recipient, based on data from TRALI patients and/or from clinically relevant experimental TRALI animal models, include sepsis, cardiac surgery, chronic alcohol abuse, shock, older age, liver surgery, history of liver disease, current smoking, higher peak airway pressure while undergoing mechanical ventilation, positive

Table 11.3 Comparison table to assist with pulmonary reaction classification.

	TRALI type 1	TRALI type 2	ARDS	TRALI and/or TACO	TACO	TAD
Hypoxemia	Present	Present	Present	Present	May be present but not required	May be present but not required
Imaging evidence of pulmonary oedema	Documented	Documented	Documented	Documented	May be present but not required	May be present but not required
Onset within 6 hours	Yes	Yes	Yes	Yes	Yes*	No*
ARDS risk factors	None	Yes – with stable or improving respiratory function in prior 12 hours	Yes – with worsening respiratory function in prior 12 hours	None, or if present, with stable or improving respiratory function in prior 12 hours	Not applicable	Not applicable
LAH†	None/mild	None/mild	None/mild	Present or not evaluable	Present	May be present but not required

* Some definitions of TACO allow onset up to 12 hours post transfusion. However, our current recommendation is that 6 hours be used. If pulmonary oedema occurs more than 6 hours following the transfusion and is clinically suspicious for a temporal association with transfusion, the case should be classified as TAD, as is currently done in many haemovigilance systems.

† LAH is difficult to assess. When LAH is suspected, we recommend using objective evaluation to determine if it is present. Objective criteria include imaging (e.g. echocardiography) or invasive measurement (e.g. pulmonary artery catheter pressure measurement). However, clinical judgement is often required and, if this is needed, should be used for case classification as follows:

- TRALI and/or TACO: Respiratory insufficiency at least partially explained by hydrostatic lung oedema resulting from cardiac failure or fluid overload, or unable to fully assess the contribution of hydrostatic lung oedema resulting from cardiac failure or fluid overload.
- TACO: Respiratory insufficiency explained by hydrostatic lung oedema resulting from cardiac failure or fluid overload.

ARDS, acute respiratory distress syndrome; LAH, left-atrial hypertension; TACO, transfusion-associated circulatory overload; TAD, transfusion-associated dyspnoea; TRALI, transfusion-related acute lung injury.
Source: Adapted from Vlaar et al. 2019 [2].

Table 11.4 Classification of pulmonary oedema not fulfilling transfusion-related acute lung injury (TRALI) criteria.*

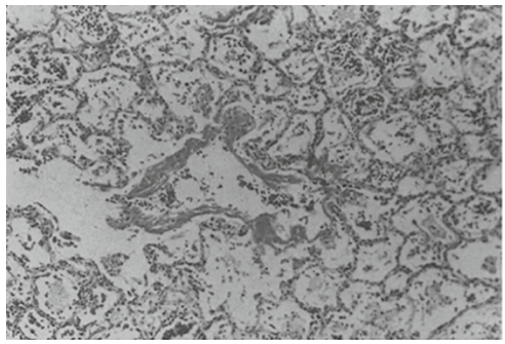
Patients who have risk factors for ARDS, and deteriorate not due to transfusion, but as a consequence of the already present ARDS risk factors	
ARDS	a. Onset of ARDS within 6 hours after transfusion, but respiratory status was deteriorating in the 12 hours prior to transfusion
	b. Preexisting ARDS of any severity that further deteriorates after transfusion, where respiratory status was already deteriorating in the 12 hours prior to transfusion
Patients in whom TRALI cannot be distinguished from TACO or in whom both conditions occur simultaneously	
TRALI /TACO cannot be distinguished	a. Clinical findings compatible with TRALI and with TACO and/or lack of data to establish whether or not significant left-atrial hypertension is present

* If pulmonary oedema occurs more than 6 hours following the transfusion, and is clinically suspicious for temporal association with transfusion, the case should be classified as transfusion-associated dyspnoea (TAD), as is currently done in many haemovigilance systems.
ARDS, acute respiratory distress syndrome; TACO, transfusion-associated circulatory overload.
Source: Adapted from Vlaar et al. 2019 [2].

intravascular fluid balance, systemic inflammation, dysregulation of CD4+ T-regulatory cells or dendritic cells and decreased levels of interleukin (IL)-10 [19,20]. A general state of inflammation is frequently present and is reflected by increased plasma levels of the pro-inflammatory cytokines IL-6 and IL-8, the latter being a potent neutrophil chemoattractant [19]. This may explain why sepsis is a patient risk factor for the onset of TRALI. In addition, increased plasma levels of the acute-phase protein C-reactive protein (CRP), a biomarker for acute infections and inflammation, is a first hit in TRALI, as CRP levels were found to be elevated in TRALI patients, and furthermore CRP was shown to functionally enhance TRALI in a murine model of antibody-mediated TRALI [21]. Furthermore, loss of immune tolerance by dysregulated CD4+ T-regulatory cells or dendritic cells was found to increase the susceptibility to antibody-mediated TRALI in murine models, and this response was associated with low plasma levels of the anti-inflammatory cytokine IL-10 [22], and indeed low plasma IL-10 levels were also found in TRALI patients in contrast to patients suffering from other pulmonary transfusion reactions. Taken together, several recipient lesions (first hit) can create susceptibility for TRALI reactions after transfusion (second hit), and this first hit is often elicited by a signature characterised by an increase in pro-inflammation in combination with a decrease in anti-inflammation.

Second-hit factors in the transfused blood product, based on clinical data and/or from clinically relevant experimental TRALI animal models, include anti-leucocyte and/or endothelium-reactive antibodies, or other soluble factors, collectively termed biological response modifiers (BRMs) (Table 11.5) [19,20]. In so-called antibody-mediated TRALI, the antibodies may be directed against cognate human leucocyte antigens (HLA) class I or II or human neutrophil antigens (HNA; i.e. a corresponding matching antigen) on recipient leucocytes or on the pulmonary endothelium. The two-hit model is supported by animal experimental evidence as well as retrospective clinical studies demonstrating that most transfused blood products containing HLA antibodies do not cause TRALI, even if a cognate recipient antigen is present. In addition, a recent study comparing TRALI-inducing to TRALI-resistant antibodies suggested that specific antibody characteristics may be critical for the TRALI-inducing effect, such as the ability of

(a)



(b)

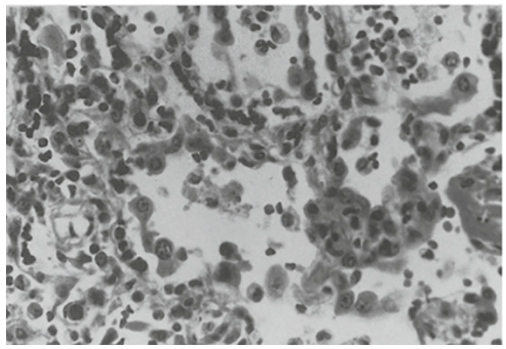


Figure 11.2 Thin sections of fixed lung from a patient with transfusion-related acute lung injury (TRALI). There is acute diffuse alveolar damage with intra-alveolar oedema and haemorrhage. Magnification (a) ×40, (b) ×440. *Source:* Silliman et al. The association of biologically active lipids with the development of transfusion-related acute lung injury. Transfusion 1997;**37**:719–26]. Reproduced with permission of John Wiley & Sons.

the antibody to activate the complement cascade [23]. In so-called non-antibody-mediated TRALI, BRMs present in cell-containing blood products may include bioactive lipids, mitochondrial damage–associated molecular patterns, aged cellular blood products and extracellular vesicles. Further involvement and elucidation of mechanistic modes of action of these BRMs are required and are the subject of ongoing research efforts. The proportion of TRALI cases caused by antibodies versus BRMs varies by the type of component transfused, with antibody-mediated mechanisms explaining the majority of cases due to plasma and non-antibody BRMs being responsible for most cases from red blood cell and platelet transfusion. Antibody mediation has previously been suggested to be responsible for around 80% of the TRALI cases; however, in the current era of TRALI risk-mitigation strategies for plasma, the percentage of antibody-mediated TRALI has relatively decreased, and the percentage of non-antibody-mediated TRALI thus relatively increased.

Susceptibility Hit and Potential Role for Perioperative Third Hit

Based on a clinically relevant experimental animal model of antibody-mediated TRALI, it has been suggested that the composition of the gut microbiota may drive the susceptibility to the first and second hits in the development of TRALI. It was demonstrated that mice housed in a more sterile environment (specific pathogen free) were resistant to antibody-mediated TRALI, whereas mice that were housed in less sterile conditions (barrier-free mice) were sensitive to development of antibody-mediated TRALI [24]. The composition of the gut microbiota differed between the resistant and sensitive mice, and treatment with broad-spectrum antibiotics depleted the gut microbiota and prevented development of TRALI in barrier-free housed mice. Furthermore, faecal transplants from TRALI-susceptible mice into TRALI-resistant mice restored the TRALI response [24]. This indicates that the gut microbiota is a so-called susceptibility hit; however, the exact interplay between the gastrointestinal flora and TRALI induction will need to be further researched.

Apart from the classic two hits of TRALI, in a perioperative setting a role may be present for a potential additional third hit that synergises or at a minimum contributes additively to both the first and second hits [25]. This third hit reflects surgical procedurally related factors, such as increased frailty associated with surgery, increased risk due to surgery and cardiopulmonary bypass. These surgical procedures are known to promote inflammation via triggering the release of

pro-inflammatory cytokines due to incisions, or pulmonary endothelial trauma due to minimally invasive procedures involving wires and cannulas, negative pressure pulmonary oedema, or extracorporeal circuit use such as cardiopulmonary bypass [25]. Overall, this third hit may, at least in part, contribute to higher incidences of TRALI in transfused surgical and/or critically ill patients. Further research is needed to establish the role of a third hit in TRALI.

All described hits in the pathophysiology of TRALI, based on clinical data and/or on clinically relevant experimental animal models, are summarised in Table 11.5.

Immune Cell Involvement

Upon the execution of the first and second hits, a complex and rapid immunological reaction occurs in the transfused recipient that is not fully understood. In general, however, pathogenic and protective recipient immune cells have been identified using clinically relevant experimental animal models of TRALI [19].

Neutrophils are classically recognised as pathogenic cells in TRALI, as they accumulate in the lungs in experimental animal models of TRALI, but have also been identified to be present in the lungs of TRALI patients in autopsy reports. In addition, neutrophil depletion in experimental animal models of antibody-mediated TRALI protected mice from TRALI development [22], and neutrophil extracellular traps (NETs) have been identified in plasma and lungs of TRALI patients and mice.

Monocytes are also pathogenic cells in TRALI. In murine models, monocytes can be triggered by antibodies to induce TRALI-inducing chemokines. In patients, monocytes have been thought to be pathogenic cells in HLA class II antibody-mediated TRALI [26], and macrophage depletion prevents the development of antibody-mediated TRALI in mice. Macrophages were also shown to secrete the protein osteopontin, which was found to be critically required for antibody-mediated TRALI induction in mice via stimulation of pulmonary neutrophil recruitment [28].

The role of recipient platelets has been a matter of debate, as they have been described to be pathogenic, partially pathogenic or dispensable in antibody-mediated TRALI animal models (for a review see [27]). Overall, it is possible that platelets in the recipient may play a limited pathogenic role in TRALI.

In contrast to pathogenic cells, CD4+ CD25+ FoxP3+ T-regulatory cells and CD11c+ dendritic cells (DCs) have been shown to be key protective cells in experimental animal models of antibody-mediated TRALI, as *in vivo* depletion of these cells enhanced susceptibility to the development of TRALI. This protective effect was found to be associated with increased levels of IL-10 [22].

A research challenge remains to decipher the complicated interplay between all protective and pathogenic recipient immune cells in the development of TRALI, including neutrophils, monocytes, macrophages, platelets, red blood cells, DCs and CD4+ T-regulatory cells, to explain how these interactions result in damage to the pulmonary endothelium and to describe the relevant effector mechanisms, for example cell surface Fcγ receptors, complement, NETs and the production of reactive oxygen species [19].

Potential Therapeutic Avenues

Clinically, there are currently no specific therapies available for TRALI. However, based on clinically relevant experimental animal models of TRALI, potential therapeutic strategies have been suggested for further exploration [19,20]. The most promising therapeutic strategy to investigate may be IL-10 therapy, as administration of IL-10 both prophylactically and therapeutically (i.e. after onset of TRALI symptoms) prevented and rescued the TRALI reaction, with normalisation of pulmonary oedema and acute lung injury [22]. Caution is advised, however, as IL-10 therapy may impair host resistance to infectious

Table 11.5 Pathophysiological hits in the pathogenesis of transfusion-related acute lung injury (TRALI).

Susceptibility hit	First hit	Second hit	Perioperative third hit
Composition of gastrointestinal microbiota	• Chronic alcohol abuse • Shock • Older age • Liver surgery • History of liver disease • Current smoking • Higher peak airway pressure while undergoing mechanical ventilation • Positive intravascular fluid balance • Systemic inflammation: increased IL-6, increased IL-8, increased CRP • Dysregulation of CD4+ T-regulatory cells or dendritic cells • Decreased levels of IL-10	Antibodies (against leukocytes and/or pulmonary endothelium): • Cognate HLA class II antibody • Cognate HNA antibody • Granulocyte antibody positive by GIFT • Cognate anti-HLA class I that activates cells as shown, for example, by granulocyte aggregation *in vitro* or at least by a positive by GIFT result BMRs: • Bioactive lipids • Mitochondrial damage-associated molecular patterns • Extracellular vesicles • Aged cellular blood products	Surgical procedurally related factors: • Increased frailty associated with surgery • Increased risk due to surgery • Cardiopulmonary bypass • Higher volume of female plasma

BMR, biological response modifier; CRP, C-reactive protein; GIFT, granulocyte immunofluorescence test; HLA, human leucocyte antigen; HNA, human neutrophil antigen; IL, interleukin.

pathogens. In addition, other potentially promising strategies may include targeting osteopontin, downmodulating CRP levels, targeting reactive oxygen species or blocking the IL-8 receptors [18,20,28] – all focused on the transfused recipient. In the long run, preventive strategies may consider the blood product, but new approaches will require experimental validation. Increasing the mechanistic understanding of the TRALI pathophysiology, with consideration of all the diverse pathogenic hits, will be imperative for optimising potential therapeutic approaches.

Mitigation Strategies

Leucocyte antibodies are much more common in ever pregnant females compared to never pregnant females or males due to maternal exposure to foetal leucocyte antigens. In 2003, this led the UK to initiate a policy of obtaining transfusable plasma units predominantly from male donors, thereby avoiding the transfusion of plasma units from female donors. With this intervention, TRALI cases from fresh frozen plasma (FFP) transfusion reported to the UK Serious Hazards of Transfusion HV system decreased from 14 cases in 2003 to 1 case in 2005. Overall, 36 cases of TRALI were reported in 2003, but only 15 cases were reported in 2010 [29]. These data led to widespread international adoption of a stringent policy of not transfusing plasma from ever pregnant females. Data from multiple studies using this intervention have been aggregated in a recent meta-analysis and in a second systematic review. Both publications reported a significant reduction in TRALI risk from plasma products after the adoption of risk-reduction measures: odds ratios (95% confidence interval) of 0.62 (0.42–0.92) and 0.27 (0.20–0.38) in the two analyses [30,31].

This policy is feasible for plasma transfusion because the number of plasma units exceeds demand, except for group AB plasma. In this latter case, plasma from nulliparous female donors is preferred to plasma from multiparous donors. However, this policy is not feasible for apheresis-derived platelets, where restriction of units to male donors would seriously jeopardise the platelet supply. It is becoming more common to test previously pregnant donors for HLA antibodies and then to redirect antibody-positive donors away from all high-plasma volume donations, including platelet or plasma apheresis. At present, techniques for neutrophil antibody identification are cumbersome and have not been widely applied in screening. For non-antibody-mediated TRALI, preventive steps are more difficult and so far no preventive measures have been recommended or undertaken.

Another strategy is to use solvent detergent plasma (SDP). The risk of TRALI is reduced by dilution of the concentration of antibodies using large pools of plasma, and possibly binding of the antibody with cognate antigens in the plasma pool reducing the chance of triggering a TRALI reaction in the recipient. Initially SDP was claimed to be TRALI free, but recently the first reports have been published [32]. Finally, reverse TRALI had been thought to be mitigated after introduction of leuco-reduced blood products. However, recently three independent groups identified an important role for reverse TRALI when antibodies were not found in the donor also in the presence of leuco-reduced products [33]. Hence, these results support antibody testing in recipients of suspected TRALI cases.

Patient Management

If TRALI is suspected, the transfusion should be stopped immediately. Patient management is supportive as there are no

specific therapeutic interventions. TRALI is often severe and virtually all patients will require some form of oxygen supplementation to maintain acceptable oxygen saturations. Contemporary evidence suggests that assisted mechanical ventilation with or without intubation will be required in two-thirds or more of cases [9]. When mechanical ventilation is required, lung-protective strategies derived from general ARDS studies should be applied, with low tidal volume ventilation (< 6 mL/kg of ideal bodyweight) and limited plateau airway pressures (< 30 cmH_2O) [33]. When mechanical ventilation is insufficient to support severe hypoxia, extracorporeal membrane oxygenation (ECMO) can be used as a last resort. There are several successful case reports applying ECMO in severe TRALI reactions. At present, there is no convincing evidence to support the use of corticosteroids. Fluid resuscitation may be required when hypotension or shock is present. However, diuretic therapy may be indicated if hypoxaemia is severe, and if the blood pressure is stable in the presence or absence of congestive heart failure or circulatory overload. Further transfusions, if needed, do not require any other special precautions, since recurrent TRALI is extremely rare.

For all suspected cases of TRALI, clinicians should obtain a chest radiograph and a blood sample that can be used for HLA phenotyping at a later time. The latter may also be used for bacterial testing if a septic transfusion reaction is under consideration. Patient testing for leucocyte antibody may be considered provided that a sample has been saved, but this is not needed if only leucocyte-reduced components have been transfused. Pre- and post-transfusion BNP or NT-BNP levels are of unclear utility when attempting to specifically distinguish TRALI from TACO.

Reporting

It is recommended that all pulmonary complications after blood transfusion should be reported to the transfusion service and then categorised (by either the transfusion service, a hospital transfusion committee or an HV system) into one of several categories: TRALI (type I or type II), ARDS, TACO, TRALI/TACO cannot distinguish, or an alternative diagnosis. Importantly, TRALI remains a clinical diagnosis and does not require detection of cognate leucocyte antibodies, though it is recommended that these data be captured through an HV reporting system.

When a suspected TRALI case is reported to the transfusion service, the medical director determines whether the case meets the diagnostic clinical criteria for TRALI. If so, the case should be reported to the blood centre so that a donor investigation is conducted; this investigation includes testing of associated donors for HLA and possibly HNA antibodies. Some laboratories in Europe also perform leucocyte crossmatching as part of the evaluation.

An 'implicated donor' is defined as one who has leucocyte antibodies that correspond to the recipient's antigen(s) or a donor whose serum is reactive against the recipient's leucocytes in a crossmatch test; this is strong evidence of antibody-mediated TRALI once the clinical diagnosis of TRALI has been made. If an implicated donor is identified, that donor should be deferred, at a minimum, from future plasma apheresis or platelet apheresis donation. Furthermore, most blood transfusion laboratories will also defer an implicated donor from whole blood donation; this is particularly true if the donor has anti-HNA-3a. It remains uncertain whether the donor should be deferred if they have leucocyte antibodies but cognate antigens are not present in the recipient, or if the crossmatch test is negative.

KEY POINTS

1) An updated international definition of transfusion-related acute lung injury (TRALI) is now available.
2) TRALI is among the leading causes of death from transfusion.
3) In the general ward, fatality occurs in 10–15% of diagnosed cases, but those who recover generally show no long-term respiratory sequelae.
4) In critically ill patients, incidence of TRALI is reported to be up to 15% and mortality may rise up to 40%.
5) The pathology of TRALI can be described by a two-hit model: first, priming of the endothelium and/or neutrophils (e.g. sepsis); and second, transfusion resulting in activation of the primed endothelium and/or neutrophils. Subsequent pulmonary oedema is influenced by recipient risk factors.
6) Substances in blood components responsible for the syndrome include leucocyte antibodies (human leucocyte antigen [HLA] and neutrophil specific) and biological response modifiers.
7) When TRALI is suspected, the transfusion should be stopped, and the case reported to the blood transfusion laboratory. Presence of HLA/HNA (human neutrophil antigen) antibodies may be supportive. However, TRALI remains a clinical diagnosis.
8) Treatment is supportive and often requires mechanical ventilation, and in extreme settings extracorporeal membrane oxygenation is needed.
9) TRALI is difficult to distinguish from transfusion-associated circulatory overload (TACO). Underlying cardiac dysfunction or evidence of fluid overload may suggest TACO.
10) Preventive measures include exclusion of blood donors implicated in TRALI cases and reducing transfusions of high-plasma-volume blood components from donors likely to possess leucocyte antibodies.
11) TRALI incidence has decreased due to minimising (or eliminating) transfusion of plasma manufactured from donations by ever pregnant female donors.
12) Reverse TRALI was thought to be eliminated through universal leuco-reduction of cell-containing blood products. Recent insights suggest that reverse TRALI is still possible in the presence of leuco-reduced products.
13) Recent studies show that SDP plasma is also able to induce TRALI in contrast to previous reports. Underreporting and underdiagnosing may be an explanation for this observation.

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12 Purported Adverse Effects of 'Old Blood'

Lirong Qu and Darrell J. Triulzi

Department of Pathology, University of Pittsburgh; Vitalant, Pittsburgh, PA, USA

Red blood cell (RBC) units are stored in a monitored refrigerator (1–6 °C) for up to 42 days in the commonly used additive solutions in the USA (AS-1, AS-3, AS-5). As standard of care, hospital blood banks/transfusion services issue the oldest available RBC units for transfusions for efficient inventory management and waste prevention. According to the most recent National Blood Collection and Utilization Survey (NBCUS) of 2017 transfusion data, 82% of RBC units were 1–35 days old and 18% were 36–42 days old at the time of transfusion among the 194 facilities that reported data for RBC age [1]. These estimates were similar to those of the 2015 NBCUS, which reported that the mean RBC storage duration at transfusion was 17.9 days. During routine RBC storage, well-characterised and well-documented biochemical, structural and metabolic changes (collectively called 'storage lesions') occur as the RBC ages [2]. It has been hypothesised that the time-dependent, *in vitro*, reversible and irreversible changes associated with the storage lesion would be associated with a deterioration of RBC quality. Therefore, the stored, aged, standard-issue RBC unit might be inferior to the freshest available RBC in achieving the optimal clinical outcomes in the transfusion recipient.

Until relatively recently, studying the impact of those *in vitro* changes on clinical outcomes of transfusion recipients has been mainly from retrospective observational reports. The literature included many observational reports asserting the adverse effects of RBC storage duration on clinical outcomes, while other reports revealed no effect of storage duration on clinical outcomes, and a few reported harmful effects of fresh units (compared with standard issue) on the clinical outcomes in transfusion recipients. There are several potential explanations for the conflicting conclusions from the mostly observational studies. First, a retrospective study design does not control for known or unrecognised, potentially clinically important factors such as baseline patient characteristics, underlying disease, the presence of co-morbidities (i.e. the need for RBC transfusion), volume transfused, transfusion of other blood components and follow-up period. Sicker patients receive more blood transfusions and thus have a greater likelihood of receiving at least one older unit, creating confounding that is difficult to address in non-randomised studies. An observational study cannot determine whether worse outcomes are due to the need for transfusion or transfusion itself (confounding by indication). Second, varied mortality (e.g. 7 day vs 28 day) and morbidity endpoints (outcomes) were reported among studies, making comparisons difficult. Third, various definitions of RBC storage

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durations were used to define 'fresher' versus 'older'. This became especially problematic when multiple units of various storage duration were transfused. Most importantly, there is no clinical evidence to support any of these definitions. Investigators have tried to extrapolate the *in vitro* changes to RBC as equivalent to *in vivo* effectiveness when defining age of RBC storage. This approach does not account for the fact that the kinetics of the *in vitro* changes are highly variable depending on the parameter and none has been shown to be clinically relevant. Fourth, varied red cell preparations with different storage media or modifications such as leuco reduction were used in studies over the years.

As a result, conclusions from these observational studies, which represent the body of literature on the subject, are conflicting. The lack of clarity from the observational studies has prompted the design and conduct of large-scale multicentre, properly powered, randomised controlled trials (RCTs) to generate robust data to resolve the issue. The data from several large-scale RCTs have recently became available, and this chapter will focus on the results from these RCTs.

Randomised Controlled Trials of Red Blood Cell Storage Duration

The first prospective RCT of RBC storage was carried out over three decades ago and involved 237 patients randomised to receive two units of either fresh whole blood (< 12 hours) or stored blood (2–5 days) at the end of the extracorporeal circulation in primary coronary bypass operation. There were no differences in postoperative bleeding, coagulation tests or transfusion requirements between the two groups [3]. Kor and colleagues performed a double-blind, single-centre randomised trial of 100 mechanically ventilated patients in the intensive care unit (ICU) to compare the effect of fresher RBCs (median age 4 days) with standard RBCs (median age 26.5 days) [4]. There was no significant difference in the primary outcome of pulmonary function assessed by the partial pressure of arterial oxygen to fraction of inspired oxygen concentration ratio, as well as the immunological and coagulation status between the two groups. Similar mortality was noted between recipients receiving fresher RBCs and those transfused with standard-issue RBCs, but the study was not powered for this outcome [4]. Several other small, single-centre, prospective randomised trials were conducted, but these studies were underpowered (Table 12.1) [5–7].

Recent Randomised Controlled Trials of Red Blood Cell Storage Duration in Children

Recently, results from multiple large RCTs have been published (Table 12.1). Three studies were conducted in paediatric patients [8–10]. The Age of Red Blood Cells in Premature Infants (ARIPI) study evaluated 377 premature infants with birthweight less than 1250 g in a neonatal ICU requiring at least one RBC transfusion [8]. A total of 188 patients received fresh RBCs (median storage 5.1 days; standard deviation [SD] 2.0), and 189 patients received RBC stored according to standard of care (median storage 14.6 days; SD 8.3). The relative risk (RR) was 1.00 (95% confidence interval [CI] 0.82–1.21) for the primary outcome of the study, a composite measure of necrotising enterocolitis, retinopathy of prematurity, intraventricular haemorrhage, bronchopulmonary dysplasia and death. The rate of clinically suspected infection was 77.7% and 77.2% for the fresher RBC group and the standard RBC group, respectively (RR 1.01; 95% CI 0.90–1.12). The rate of positive cultures was 67.5% and 64.0% for the fresher RBC group and the standard RBC group, respectively (RR 1.06; 95% CI 0.91–1.22) [8]. So here the use of fresher RBCs compared with standard of care did not decrease or increase the rate of complications or death in this population of premature, very low

Table 12.1 Randomised controlled trials (RCTs) of red blood cell (RBC) storage duration.

Author (ref) year (trial name)	Study design	Definition of storage duration	Clinical setting	No. of patients	Adverse effects with longer storage?
Cooper [15] 2017 (TRANSFUSE)	Multicentre RCT	Freshest vs standard issue	Intensive care unit (ICU)	4994	No for 90-day mortality
Heddle [13] 2016 (INFORM)	Multicentre RCT	Freshest vs standard of care	Hospitalised patients	31 497	No for mortality
Steiner [12] 2015 (RECESS)	Multicentre RCT	≤ 10 days vs ≥ 21 days	Cardiac surgery	1098	No for changes in organ dysfunction No for 7-day and 28-day mortality
Lacroix [11] 2015 (ABLE)	Multicentre RCT	< 8 days vs standard issue	ICU	2430	No for 90-day mortality No for all secondary outcomes
Spinella [10] 2019 (ABC-PICU)	Multicentre RCT	≤ 7 days vs standard of care	Paediatric ICU	1538	No for organ dysfunction No for incidence of sepsis
Dhabangi [9] 2015 (TOTAL)	Single-centre Randomised non-inferiority	8 days (7–9) vs 32 days (30–34)	Paediatric acute care centre	290	No for reduction of elevated blood lactate levels
Fergusson [8] 2012 (ARIPI)	Multicentre RCT	< 7 days vs standard of care	Premature infants	377	No for mortality No for rate of complications
Wasser [3] 1989	Single-centre RCT	< 12 hours vs 2–5 days	Cardiac surgery	237	No for coagulation rests, postoperative bleeding or transfusion requirements
Kor [4] 2012	Single-centre RCT	≤ 5 days vs standard-issue RBCs (median 21 days)	ICU	100	No difference in pulmonary function
Fernandes [5] 2001	Single-centre RCT	Continuous variable	ICU (sepsis)	15 (10 transfused; 5 received albumin)	No for gastric mucosal pH
Walsh [6] 2004	Single-centre RCT	≤ 5 days vs ≥ 20 days	ICU (on mechanical ventilation)	22	No for gastric mucosal pH
Yuruk [7] 2013	Single-centre	< 1 week vs. 3–4 weeks	Haematology patients	20	Same increase in perfused vessel density in both groups

birthweight neonates [8]. This study was limited by the fact that the age of blood in the standard-of-care group was only 14 days.

The Tissue Oxygenation by Transfusion in Severe Anemia with Lactic Acidosis (TOTAL) study evaluated whether longer-storage RBC units were inferior to shorter-storage RBC units for tissue oxygenation (measured by reduction in blood lactate levels and improvement in cerebral tissue oxygen saturation) among 290 children between 6 and 60 months of age with lactic acidosis due to severe anaemia, mostly secondary to malaria or sickle cell disease [9]. Median (interquartile) storage duration for RBC units was 8 (7–9) days for shorter storage versus 32 (30–34) days for longer storage. There was no statistical difference between the two groups at 0, 2, 4, 6, 8 or 24 hours in mean lactate levels or in lactate reduction. Other outcomes such as clinical assessment, cerebral oxygen saturation, adverse events, electrolyte abnormality and survival were not significantly different between the groups [9]. The study demonstrated that transfusion of longer-storage RBC units did not result in inferior reduction of elevated blood lactate levels compared with shorter-storage RBC units among children with lactic acidosis due to severe anaemia.

The Age of Blood in Children in Pediatric Intensive Care Unit (ABC-PICU) study randomised 1538 critically ill children (age 3 days to 16 years; median age 1.8 years) to receive either fresh (stored ≤ 7 days) or standard-care RBC units, which involved transfusing the oldest compatible unit available in the transfusion service on that day [10]. The median age of transfused RBCs was 5 days (interquartile range 4–6 days) in the fresh group and 18 days (interquartile range 12–25 days) in the standard-issue group. There was no significant difference in the primary outcome of organ dysfunction (20% in the fresh group vs 18% in the standard-care group; 95% CI –2.0% to 6.1%; p = 0.33). The prevalence of sepsis was 25.8% (160 of 619) in the fresh group and 25.3% (154 of 608) in the standard-care group. The study concluded that the use of fresh RBCs did not reduce the incidence of new or progressive organ dysfunction compared with standard-issue RBCs among critically ill paediatric patients.

Recent Randomised Controlled Trials of Red Blood Cell Storage Duration in Adults

Several large-scale RCTs in adults have also reported their findings (Table 12.1). The Age of Blood Evaluation (ABLE) study randomised 2430 ICU patients to receive RBCs that had been stored for less than 8 days or standard-issue RBCs (the oldest compatible units available in the blood bank) [11]. A total of 1211 patients received fresh RBCs (mean age 6.1 days; SD 4.9) and 1219 patients received standard-issue RBCs (mean age 22 days; SD 8.4; p < 0.001). The primary endpoint of 90-day mortality occurred in 37.0% in the fresh-blood group and 35.3% in the standard-blood group (95% CI for the absolute difference –2.1 to 5.5). There were no significant differences in any of the secondary outcomes (major illness; duration of respiratory, hemodynamic or renal support; infection; length of hospital stay; and transfusion reactions) between the two groups [11].

The Red Cell Storage Duration Study (RECESS) randomised 1096 patients undergoing cardiac surgery to receive RBCs stored for 10 days or less (shorter-term storage group) or for 21 days or more (longer-term storage group) [12]. The mean (±SD) duration of storage was 7.8±4.8 days in the shorter-term storage group and 28.3±6.7 days in the longer-term storage group. No difference was seen in the median composite change in Multiple Organ Dysfunction Score (MODS) at day 7 (8.5 vs 7.5 points for shorter-term and longer-term storage groups, respectively; p = 0.44). The 7-day mortality was 2.8% in the shorter-term storage group and 2.0% in the longer-term storage group (p = 0.43). The all-cause mortality

rate at 28 days was 4.4% and 5.3%, respectively (p = 0.57). The study concluded that RBC storage duration was not significantly associated with day-7 changes in MODS, serious adverse events or 28-day mortality rate among patients undergoing cardiac surgery [12].

The Informing Fresh versus Old Red cell Management (INFORM) trial randomised a general population of hospitalised adults (n = 31 497) to receive either the freshest RBCs available in the inventory (short-term storage group) or the oldest available RBCs (long-term storage group) [13]. Analysis was performed on 20 858 patients with type A or O blood group: 6936 patients received RBCs stored for a mean duration of 13.0 day (short-term storage group); 13 922 patients received RBCs stored for a mean of 23.6 days (long-term storage group). There was no significant difference in the rate of death between the two groups: 9.1% (634/6936) in the short-term storage group; 8.7% (1213/13922) in the long-term storage group (odds ratio 1.05; 95% CI 0.95–1.16; p = 0.34). The analysis of 24 736 patients with any blood type found similar rates of death of 9.1% and 8.8%, respectively (odds ratio 1.04; 95% CI 0.95–1.14; p = 0.38) [13]. The study indicated that there was no mortality benefit in transfusing the freshest available RBC units compared with the standard practice of transfusing the oldest available RBC units in a general hospital patient population.

A secondary analysis of the INFORM trial evaluated whether increased risk of in-hospital mortality was associated with transfusing RBC units stored for more than 35 days [14]. Among 24 736 eligible patients, 4480 patients (18%) were exposed to RBCs with the longest storage (> 35 days), 1392 patients (6%) were exposed exclusively to RBCs with the shortest storage (≤ 7 days) and 18 854 patients (76%) were exposed to RBCs stored for 8–35 days. Transfusion of RBCs stored for longer than 35 days was not associated with increased risk of in-hospital death compared with transfusion of the freshest RBCs (stored ≤ 7 days) – in patients with blood group A or O: hazard ratio (HR) 0.94, 95% CI 0.73–1.20, p = 0.60; in all patients: HR 0.91, 95% CI 0.72–1.14, p = 0.40). In addition, the risk of in-hospital death did not differ between patients transfused with RBCs stored for 8–35 days and patients receiving RBCs stored for 7 days or fewer – in patients with blood group A or O: HR 0.92, 95% CI 0.74–1.15, p = 0.60; in all patients: HR 0.90, 95% CI 0.73–1.10, p = 0.29) [14]. Therefore, among the general hospitalised patient population, exposure to RBCs stored for over 35 days (during the last week of RBC storage) was not associated with increased risk of in-hospital mortality compared with exposure exclusively to the freshest RBCs.

The Standard Issue Transfusion versus Fresher Red-Cell Use in Intensive Care (TRANSFUSE) trial compared the effects of the freshest available RBCs (short-term storage group) with those of standard-issue (oldest available) RBCs (long-term storage group) in 4994 critically ill adults [15]. The 90-day mortality was 24.8% (610/2457) for the short-term storage group (mean storage 11.8 days) and 24.1% (594/2462) for the long-term storage group (mean storage 22.4 days), with an absolute risk difference of 0.7% (95% CI –1.7 to 3.1; p = 0.57). The 180-day mortality difference between the two storage groups was 0.4% (95% CI –2.1 to 3.0; p = 0.75). Most of the prespecified secondary measures showed no significant difference in outcomes between groups [15]. This study provided additional evidence supporting that even critically ill patients do not benefit from the freshest RBCs compared with the standard practice of using the oldest stored RBCs.

Another RCT was designed to test the effect of RBC storage age on outcomes in transfused cardiac surgery patients [16]. The trial was stopped at its midpoint owing to enrolment constraints; the data did not support either the efficacy or the futility of transfusing either younger or older RBC units [16].

Systematic Reviews and Secondary Analyses of Randomised Controlled Trials of Red Blood Cell Storage Duration

A recent systematic review and meta-analysis of 16 RCTs including 31 359 patients showed that transfusion with fresher compared with older RBCs was not associated with risk of death (RR 1.04, 95% CI 0.98–1.09; p = 0.20; I^2 = 0%; high-quality evidence), but was associated with a higher risk of transfusion reactions (RR 1.35, 95% CI 1.04–1.76; p = 0.02; I^2 = 0%; high-quality evidence) and infection (RR 1.08, 95% CI 1.00–1.17; p = 0.05; I^2 = 0%; moderate evidence). Therefore, transfusion of fresher RBCs is not associated with decreased risk of death, but is associated with higher rates of transfusion reactions and possibly infection [17]. A previous meta-analysis of 12 RCTs that enrolled 5229 patients also showed little or no impact of fresher versus older RBCs on mortality (mortality [RR] 1.04; 95% CI 0.94–1.14) or on adverse events (RR 1.02; 95% CI 0.91–1.14). A small increase in risk of infection was also identified in patients receiving fresher RBC units in a meta-analysis of randomised trials comparing RBCs of different storage ages (RR 1.09; 95% CI 1.00–1.18) [18].

Two secondary analyses of previously published RCTs have reported adverse effects of stored RBCs in patients who received many RBC units. A secondary analysis of the ABLE trial observed a dose-dependent relationship between stored RBCs and mortality, with an increased risk of 90-day and 180-day mortality (but not earlier endpoints) in patients receiving more than six units of stored RBCs [19]. The hazard ratio for death by 90 days was 0.55 (95% CI 0.11–0.98, fresh vs. standard) after exposure to six RBC units, but 1.45 (95% CI 1.06–1.98) after exposure to one unit, indicating that patients transfused with only a single standard-issue RBC unit had a lower risk of death by 90 and 180 days compared with those transfused with a single fresh RBC unit. In addition, the main analysis of the ABLE trial included a subgroup analysis of patients who received no more than three RBC units and more than three RBC units and found no significant difference between groups. The RECESS study found no difference between subgroups that received fewer than eight versus eight or more RBC units. A secondary analysis of the Pragmatic, Randomised Optimal Platelet and Plasma Ratios (PROPPR) trial reported that older RBC units are associated with an increased risk of 24-hour death in trauma patients receiving massive transfusions (≥ 10 units), but not in those receiving fewer than 10 units [20]. These studies suggest that exposure to larger numbers of longer-stored red cells may be associated with worse clinical outcomes, but the inherent limitations of secondary analyses require that these results be interpreted with caution.

With a total of more than 30 000 patients studied in the RCTs (Table 12.1), not a single trial demonstrated significant differences in clinical outcomes between the stored RBC units and fresh RBC units in its primary analysis. Thus, these large RCTs provide robust consensus data and show no differences in clinical outcomes associated with the duration of RBC storage. Although these clinical trials did not specifically address the effects of RBC units transfused at the extreme end of their storage spectrum ('very fresh' or 'very old'), i.e. RBCs stored for fewer than 5 days or more than 37 days (38–42 days old), or specific patient subgroups such as those with massive transfusions who were exclusively receiving the 'very fresh' or 'very old', the results are applicable to the great majority of the general medical/surgical patient population. The AABB (Association for the Advancement of Blood and Biotherapies) has issued evidence-based guidelines concluding that 'patients should receive RBC units selected at any point within their licensed dating period (standard issue) rather than limiting patients to transfusion

of only fresh (storage length: <10 days) RBC units (strong recommendation, moderate quality evidence)' [21].

Future Research

The wide agreement of RCT data indicating that storage duration does not impact clinical outcomes has led investigators to assess RBC quality using alternative methods. The REDS-III NHLBI RBC-OMICS study performed red cell haemolysis studies at end of storage and related the findings to genome-wide association study (GWAS) analysis in more than 13 000 ethnically diverse blood donors [22]. Others have not only applied proteomics and metabolic analyses to blood storage duration, but also addressed other variables such as blood preparation methods and additive solutions [23,24]. The 'omics' approach to the study of red cell components provides a sophisticated glimpse into the complex changes in RBC components that go far beyond storage duration and implicate donor genetics as well as external factors such as donor behaviours, additive solutions and processing methods, which may all play into red cell quality. It is possible that units collected from donors with some genetic traits, such as G6PD, or conceivably from donors with a combination of traits, may have storage lesions with clinically significant effects, although the significance of putative effects has not been demonstrated (see [25]).

Conclusions

Mounting evidence from multiple RCTs has provided strong indications that the storage duration of RBCs does not have measurable adverse effects on the clinical outcomes of transfusion recipients in a variety of clinical settings, which include premature infant, paediatric ICU, adult cardiac patients, adult ICU and general hospitalised patients. Fresher RBCs did not produce better clinical outcomes than stored, aged, older (or standard-issue) RBCs in the transfused patient populations studied. Therefore, the current transfusion practice of issuing the oldest available units for transfusion is deemed safe. Although the effects of RBCs stored at the extreme ends of the storage spectrum (i.e. 'very fresh' or 'very old') were not evaluated due to the feasibility issues, the clinical applications of such studies would also be very limited. Taken together, data from the RCTs support the current transfusion practice of issuing the oldest available units for transfusion and the duration of *in vitro* RBC storage appears to have very little clinical relevance in the majority of clinical encounters. Future approaches to assess red cell quality using 'omics' methods hold promise in defining the optimal-quality RBC units to inform a precision transfusion medicine strategy.

KEY POINTS

1) Historical observational reports suggested adverse effects of red blood cell (RBC) storage duration on clinical outcomes, although such analyses had many confounding factors.
2) Multiple large-scale randomised controlled trials (RCTs) have provided convincing data showing that duration of RBC storage is not associated with adverse clinical outcomes in transfusion recipients in a variety of clinical settings.
3) With a total of more than 30 000 patients studied in the RCTs, not a single trial demonstrated significant differences in clinical outcomes between the stored RBC units and fresh RBC units in its primary analysis.
4) The current transfusion practice of issuing the oldest available RBC units for transfusion is deemed safe.
5) Future research will examine the genetic and manufacturing contributions to RBC quality using genetics, proteomics and metabolic analyses, and the potential pathophysiological effects of aged cells and RBC microparticles.

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13 Transfusion-Induced Immunomodulation

Neil Blumberg, Majed A. Refaai and Joanna M. Heal

Department of Pathology and Laboratory Medicine, University of Rochester; Blood Bank/Transfusion Service of Strong Memorial Hospital, Rochester, NY, USA

Transfusion immunomodulation refers to a constellation of complex alterations in recipient immune function that affect clinical outcomes. In theory, this includes almost all complications of transfusion other than transmission of infectious agents. Adverse events such as alloimmunisation to blood cell or plasma protein antigens, febrile non-haemolytic transfusion reactions, anaphylaxis, acute respiratory distress syndrome/transfusion-related lung injury (TRALI) and congestive heart failure (transfusion-associated circulatory overload, TACO) following transfusion have immune effects as their primary mechanisms or as components of their pathophysiology. For example, 50% of episodes of TACO are attended by fever or other inflammatory findings. This chapter will focus on immune effects and clinical events thought likely to be immune in origin, which involve transfusion's effects on innate or adaptive cellular immunity. Innate immunity broadly refers to the roles of cells such as neutrophils, lymphocytes, monocytes/macrophages and natural killer (NK) cells. Adaptive immunity includes humoral immunity (primarily B cells and antibodies) and cellular immunity (primarily T and dendritic cells mediating cellular cytotoxicity). Clinical outcomes potentially influenced by immunomodulation include increases in postoperative infection, cancer recurrence, metastasis or mortality, as well as facilitation of organ allograft acceptance, inflammatory changes such as systemic inflammatory response syndrome/sepsis and multi-organ failure (including lung injury), necrotising enterocolitis of the newborn and enhanced success of pregnancies in patients with repetitive spontaneous abortions.

Adverse events mediated by adaptive humoral immunity (i.e. antibodies that cause red cell destruction, TRALI, anaphylaxis and allergic reactions, platelet refractoriness, posttransfusion purpura, neonatal alloimmune thrombocytopenia, organ allograft rejection, etc.) will be discussed in other chapters.

The history of transfusion immunomodulation as a comprehensive theory addressing blood transfusion's profound influence on immunity antedates our current knowledge and models of adaptive immunity (primarily T cells) and innate immunity as distinct aspects of immune function. In the 1960s and 1970s, when lymphocytes were still considered cells of uncertain function, observational data demonstrated that renal failure patients receiving haemodialysis who required red cell transfusions had better renal allograft survival after transplantation than did patients who were not transfused [1].

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This enhancing effect was reproducible, dose dependent, demonstrable in animal models and more pronounced in patients receiving whole blood or unmanipulated red cells, compared with patients receiving relatively leucocyte-reduced, washed or frozen/deglycerolised red cells. Animal models demonstrated that allogeneic leucocytes, and perhaps platelets, could mediate these effects, but not allogeneic plasma. The concept that transfusion was 'immunosuppressive' grew out of these clinical observations, which have never been validated in randomised clinical trials. One lesson from this story is that important and clinically useful and actionable findings can derive from clinical cohort studies and observational data, although randomised trials are a definitive proof that is usually required.

Almost two decades later, animal model investigators Brian Shenton and colleagues in Newcastle, UK, reported that allogeneic blood transfusions were associated with more rapid tumour growth in animals [2]. Paul Tartter and Lewis Burrows, surgeons in New York City, observed that colorectal cancer recurrence was more frequent in transfused patients [3]. The broad field of transfusion immunomodulation research thus began in the early 1980s. Shortly after, Tartter and colleagues noted that postoperative nosocomial infections were strikingly more frequent in transfused patients undergoing colorectal surgery [4]. Our centre later reported that this increase in postoperative nosocomial infections after surgery was not seen in patients receiving autologous transfusions. Furthermore, a role for stored supernatant plasma was proposed due to the association of whole blood with increased cancer recurrence. These findings supported an immune allogeneic mechanism underlying adverse clinical outcomes [5,6].

Further animal model investigations from Cincinnati (Wesley Alexander and colleagues) [7] and San Antonio (Paul Waymack and colleagues) [8] demonstrated that promotion of infection was seen when allogeneic, as opposed to syngeneic, transfusions were given. This is key evidence that these phenomena are indeed immune mediated. More recently, randomised trials of autologous [9], leucocyte-reduced [10] and restrictive red cell transfusions [11] demonstrated that each intervention is associated with clinically relevant reductions in nosocomial postoperative infections. Furthermore, observational data have shown that allogeneic platelet transfusions are associated with increases in multi-organ failure and recurrence of acute leukaemia. There are preliminary data that removing stored supernatant from transfused red cells and platelets by washing may mitigate pro-inflammatory/immunomodulatory effects in both the surgical and haematology settings.

In recent decades, it has been postulated that the red cell storage lesion may account for the adverse events seen in transfused patients. Studies have failed to find a benefit of transfusing shorter-storage red cells, and many have assumed that this disproves the existence of transfusion immunomodulation, which is an erroneous assumption. There are a number of reasons that this assumption is incorrect, most of all that there is an extensive literature demonstrating *in vitro*, in animal models and in randomised trials that allogeneic transfusions alter recipient immune function and the trajectory of posttransfusion clinical events. In addition, the randomised trials of red cell storage duration could not directly compare the effects of the longest versus shorter storage periods.

None of the trials allowed for the possibility that the freshest red cells are the most toxic to the recipient, for which there is evidence in randomised trials. A meta-analysis of trials of storage duration found a higher incidence of posttransfusion nosocomial infection in the shorter-storage study arms [12]. Finally, these studies did not account for the immunomodulatory effects of plasma and platelet transfusions, which were quite common in both study arms of the red cell storage duration trials. Indeed, the immunomodulatory effects of plasma, platelets and cryoprecipitate are only now being studied in detail.

In this chapter, we will review the randomised trials that have definitively demonstrated that transfusion causes increases in nosocomial infection after surgery, because these effects can be partially mitigated by leucocyte reduction, autologous transfusion and restrictive transfusion practices. Then, we will summarise other effects based upon observational and cohort studies, as well as animal models. The proposed immunological mechanisms underlying these findings, inferred from *in vitro* and animal model experiments, will be summarised. The question of storage age of red cells as mediators of transfusion immunomodulation will be addressed, along with future studies needed to better understand the mechanisms of transfusion immunomodulation and further mitigate the immunological toxicity of allogeneic transfusions.

Mitigation of Immunomodulation after Red Cell Transfusion

For some decades, the very existence of transfusion immunomodulation has been questioned. This raises the issue of how we establish causal associations between transfusion and altered clinical outcomes. Typical criteria supporting a causal association include reproducibility, dose dependence, mechanistic rationales and the ability to modify the association by altering the exposure to the proposed cause of the effect. While it is often stated that the strongest evidence for transfusion immunomodulation is that allogeneic transfusions improve renal allograft survival, this is not the case. Although evidence that transfusions benefit recipients of renal allografts by enhancing graft survival is strong, it is limited to mechanistic studies, animal models and epidemiological and cohort controlled trials in which patients have been selected to receive or not receive allogeneic red cells and renal allograft survival determined.

Importantly, there is conclusive evidence that the association between allogeneic red cell transfusion and postoperative infection is causal. There are mechanistic studies demonstrating that allogeneic leucocytes mediate downregulation of host defences. Animal model studies and cohort/epidemiological data demonstrated a dose-dependent effect of red cell transfusions on postoperative infections, independent of other prognostic factors. However, the strongest and most indisputable evidence that allogeneic red cell transfusions cause increased postoperative infections, likely via an immunological mechanism, comes from randomised trials in which patients undergoing surgery have received either leucocyte-reduced or non-leucocyte-reduced red cells, autologous versus allogeneic red cell transfusions, or have been treated with restrictive versus liberal red cell transfusion thresholds. Meta-analyses of these randomised trials confirmed that patients in the leucocyte-reduced, autologous or restricted transfusion arms experienced fewer nosocomial infections than patients in the non-leucocyte-reduced, allogeneic or liberal red cell transfusion threshold arms. Removing most allogeneic leucocytes, employing autologous red cell transfusions (which replace or minimise allogeneic red cell transfusions) or reducing the dose of allogeneic red cells through restrictive thresholds all lead to reductions in postoperative infections (Figures 13.1–13.3). These effects are likely to be clinically significant, as the risk of infection is approximately 55% less in patients receiving leucocyte-reduced red cells and reductions in infection of a similar order of magnitude are seen in those receiving autologous transfusions compared to allogeneic transfusions or those transfused using restrictive compared to liberal transfusion thresholds.

While some early meta-analyses of leucoreduction trials, involving multiple publications from one group, claimed there was no evidence for reductions in postoperative infections with leuco-reduced transfusions, multiple subsequent meta-analyses [10,13–15]

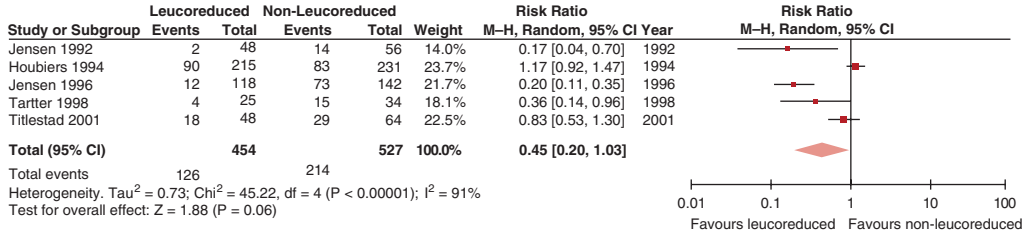

Figure 13.1 Summary of the meta-analysis of randomised trials of leucocyte-reduced red cell transfusions to mitigate postoperative infection in colorectal cancer surgery. The risk of infection is approximately 55% less in patients receiving leucocyte-reduced red cells. *Source*: Data extracted from original papers and Blumberg 2007 [13].

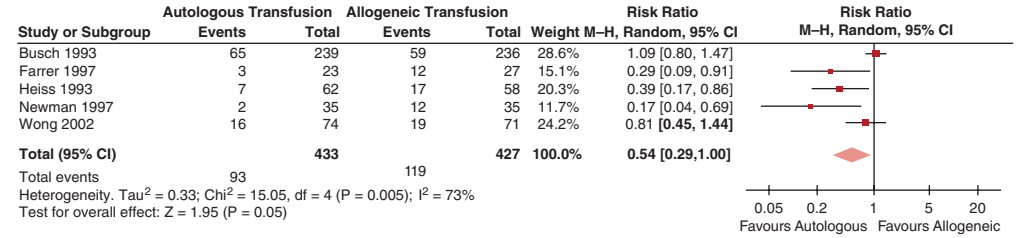

Figure 13.2 Summary of the meta-analysis of randomised trials of autologous transfusions (either pre-deposit, haemodilution or salvage) to mitigate postoperative infection after surgery. The risk of infection is approximately 46% less in patients receiving autologous red cells [9].

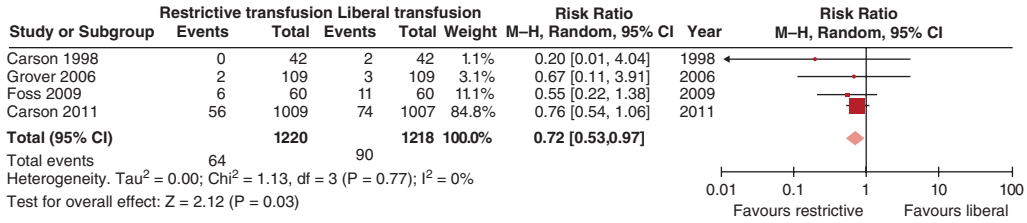

Figure 13.3 Summary of the meta-analysis of randomised trials of restrictive red cell transfusion thresholds (usually <70 g/L) to mitigate postoperative infection in orthopaedic surgery. The risk of infection is 28% less in patients receiving red cells according to restrictive criteria [16].

have found that leuco reduction is very effective at reducing posttransfusion infections and, in cardiac surgery, mortality. The likely reasons the earlier meta-analyses reached erroneous conclusions include methodological choices that eliminated studies from consideration, based upon the authors' views, included many non-transfused patients, thus favouring the null hypothesis, and employed imputed rather than actual data.

Leucocyte-Reduced Allogeneic Red Cell Transfusions Reduce Mortality in Cardiac Surgery

Although the causal effect of red cell transfusions in promoting postoperative infection might be thought of as purely immunosuppressive, it is clear that transfusion in the setting of cardiac surgery also causes increased inflammatory responses. The strongest proof

of this is that transfusion is associated in a dose-dependent fashion with multi-organ failure as well as subsequent mortality in cardiac surgery. One of the most important benefits of leucocyte-reduced red cell transfusions is that they reduce mortality after cardiac surgery [15]. While the effect of leucocyte reduction on postoperative infection is less pronounced in the setting of cardiac surgery than in colorectal surgery, it is likely that leucocyte reduction mitigates postoperative multi-organ failure, as shown in some in randomised trials. As multi-organ failure is believed to represent the effects of exaggerated inflammatory responses to apparent or occult infection, the mechanism of benefit may be somewhat similar to that seen in non-cardiac surgery settings.

Two groups in The Netherlands demonstrated a 50% reduction in mortality in cardiac surgery patients randomised to receive leucocyte-reduced transfusions. Thus, leucocyte reduction has been almost universally adopted for cardiac surgery worldwide, even in hospitals that do not practise universal leucocyte reduction. There is also evidence that leucocyte reduction, autologous transfusion and restrictive transfusion practices reduce recipient inflammatory responses as measured by cytokine levels *in vivo* or *in vitro*. Removal of stored supernatant from stored red cells by washing with normal saline further reduces the inflammatory response in cardiac surgery and in animal models. Thus, removal of stored supernatant from red cells and platelets holds promise as an additional potential strategy to minimise morbidity and mortality in heavily transfused patients. Strategies that have been proven or proposed to mitigate transfusion immunomodulation and its adverse consequences are summarised in Box 13.1. 'Proven' is used where epidemiological/observational, mechanistic and animal model data support a cause-and-effect relationship, including dose response, or that randomised trials have proven that the effect is, at least in part, causal.

Box 13.1 Strategies to mitigate transfusion immunomodulation effects

- Leucocyte reduction of red cells and platelets (proven in randomised trials) reduces postoperative infection, multi-organ failure and mortality in cardiac surgery and platelet transfusion refractoriness.
- Leucocyte reduction of transfused blood reduces inflammation, lung injury and red cell alloimmunisation in recipients by removing DNA, histones and platelets that can exacerbate innate and humoral adaptive immune responses (proposed).
- Autologous transfusion to reduce the use of allogeneic red cells in surgery reduces postoperative infection (proven in randomised trials) and may reduce postoperative thrombosis (proposed – observational data).
- Restrictive red cell transfusion practice (proven in randomised trials) reduces postoperative infection in some surgical settings (orthopaedic surgery) and may reduce multi-organ failure, thrombosis and mortality (proposed from both randomised trial and observational data).
- Washing of red cell and platelet transfusions reduces treatment-related mortality and recurrence in acute myeloid leukaemia and reduces mortality in cardiac surgery (proposed from single pilot randomised trials).
- Improve red cell viability through better storage solutions and rejuvenation prior to transfusion (proposed from animal model studies, *in vitro* mechanistic studies and observational cohort studies).
- Reduced platelet activation and dysfunction during storage through improved storage solutions/conditions to reduce the likelihood of posttransfusion inflammation and organ injury (proposed from observational studies, animal models and *in vitro* mechanistic studies).

Observational and Cohort Studies of Transfusion Immunomodulation Link Red Cell Transfusions to Both Beneficial and Adverse Outcomes

The original data supporting transfusion immunomodulation derive from cohort studies demonstrating improved renal allograft survival in transfused patients receiving red cells for anaemia caused by chronic renal failure. These patients were found to have superior renal allograft survival compared to non-transfused patients. This effect was dose dependent and was stronger when the transfusions were red cells or whole blood rather than washed or frozen/deglycerolysed red cells. This led Tartter and Burrows to propose that this immunosuppressive effect might be deleterious in cancer patients. A broad range of studies demonstrated a consistent dose-dependent association between red cell transfusion and earlier cancer recurrence or cancer-specific mortality. However, the effect was much greater in colorectal, gastric and head and neck cancers, and undetectable in some other cancers such as breast cancer. It was then found that, much like the observations in renal transplantation, red cells transfused as whole blood were much more potently associated with poor outcomes than relatively plasma- and leucocyte/platelet-depleted red cells [15,17]. This suggested a cause-and-effect relationship given the presence of immunomodulatory substances in greater quantities in whole blood.

Animal models demonstrated that leucocyte reduction might reduce promotion of cancer metastasis by transfusions, but this has not been confirmed in human trials. The few randomised trials of leucocyte reduction alone have not shown a benefit in reduced cancer recurrence, suggesting that supernatant stored plasma might play a role in this association, if causal. Autologous transfusion showed promise in one randomised trial in colorectal cancer as a strategy to reduce the adverse effects of transfusion immunomodulation on cancer recurrence. There have been no randomised trials of washed transfusions in cancer patients, except for a small pilot study demonstrating a 50% reduction in mortality in patients with acute leukaemia randomised to receive washed red cells and platelets. This was confirmed in an implementation trial in the same centre, but has not been studied otherwise.

There are observational data that allogeneic transfusion and its attendant immunomodulatory effects have beneficial effects on the recurrence of repetitive spontaneous abortion, recurrence of regional enteritis after surgical resection and a few other inflammatory diseases. However, there are also observational data that allogeneic transfusions are associated with multi-organ failure, acute lung injury, acute gut injury in premature newborns and a variety of other conditions that may involve immune dysregulation or be initiated by clinically occult nosocomial infection. Box 13.2 summarises some of these studies.

Duration of Red Cell Storage and Immunomodulation

Observational data in some centres (primarily in North America) suggested that transfusion of red cells stored for a longer period of time was associated with poorer clinical outcomes, in particular nosocomial infection, multi-organ failure, thrombosis and mortality. Animal model data confirmed these results. It is clear that red cells stored for a long time, even when leucocyte reduced, contain more free haemoglobin, haem, iron and red cell–derived microparticles. These mediators are, hypothetically, strong candidates to cause impaired host defences, thrombosis and inflammation. Nonetheless, multiple randomised trials have demonstrated that in typical clinical practice, use of shorter-storage red cells (typically 7–10 days or fewer) does not mitigate the associations

Box 13.2 Associations between immunomodulation-mediated altered clinical outcomes and allogeneic transfusions

- Enhancement of renal allograft acceptance (proven).
- Increased postoperative infections (proven).
- Increased recurrence of some solid tumours (proposed).
- Increased inflammation (proven) leading to organ failure (proven for lung) and systemic inflammatory response syndrome/multi-organ failure in surgical patients, particularly after cardiac surgery or liver transplantation (proposed).
- Increased thrombosis, perhaps through combined rheological and inflammatory mechanisms (proposed).
- Reduced repetitive spontaneous abortion in some women treated with paternal or third-party leucocytes (proposed).
- Reduced recurrence of autoimmune inflammatory conditions (regional enteritis, rheumatoid arthritis) (proposed).
- Increased reactivation of latent viral infection (proposed, but the only randomised trial in human immunodeficiency virus [HIV] failed to find any evidence).
- Increased necrotising enterocolitis in premature newborns (proposed).

between transfusion and infection, thrombosis and mortality. This may be due to the inability to compare patients receiving only very long-storage red cells (i.e. > 35 days) with those receiving shorter-storage red cells. It has also been proposed that shorter-storage red cells have altered oxidation/reduction properties, which might account for the adverse association of shorter-storage red cells with an increased risk of nosocomial infection [12]. In addition, many of these trials included patients receiving platelet and plasma transfusions, which could obscure any benefit of shorter-storage red cells.

Nonetheless, it seems likely that for most clinical settings, a few units of longer-stored red cells given to an adult patient probably do not cause clinically significantly greater immune modulation and poorer outcomes than similar small quantities of shorter-storage red cells. It may be that when the dose of longer-storage red cells is much greater, as in infants undergoing cardiac surgery, immunomodulation is more prominent. There are observational data to suggest this may be so, particularly when red cells are exposed to stresses such as irradiation and washing, as in a study by Cholette and colleagues (Figure 13.4) [19].

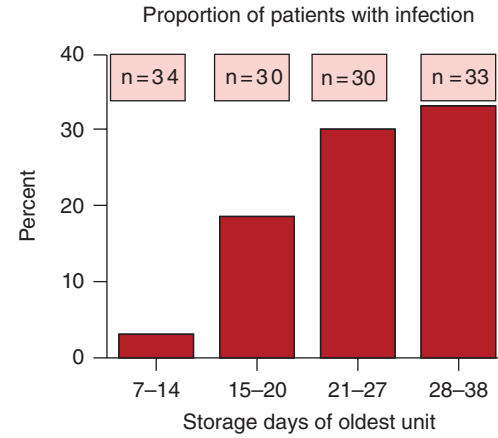

Figure 13.4 The proportion of patients with infection as a function of the oldest red cell unit transfused in a randomised trial of washed, irradiated, leucocyte-reduced red cells in paediatric cardiac surgery. The almost 10-fold difference is unlikely to be totally explained by confounding, and suggests that older red cells, when transfused at doses of hundreds of millilitres per kilogram (as in infants), may be more immunomodulatory than shorter-storage red cells, but these observational data require confirmation in a randomised trial [18].

Experimental Studies and Transfusion Immunomodulation

Allogeneic transfusions, as opposed to syngeneic transfusions, have been repeatedly demonstrated to alter outcomes in animal

models (Box 13.3). For a phenomenon as complex as transfusion immunomodulation, exact understanding of the pleiotropic effects seen may not be readily achieved. There are a plethora of *in vitro* and animal model data demonstrating a vast range of immunological changes after allogeneic transfusions or mixing experiments (Box 13.4).

All these findings are generally consonant with upregulation of humoral immunity, downregulation of cellular immunity and aberrations of innate immunity (both suppressive and inappropriately activating). These findings provide logical but largely speculative explanations for the associations of allogeneic transfusion with a wider variety of inflammatory and/or immunological reactions, including antibody formation, nosocomial infection, thrombosis, cancer recurrence, organ injury, reduced spontaneous abortions and necrotising enterocolitis in newborns. It is reasonable to suggest that induction of type 2 immune deviation (towards interleukin [IL]-10, IL-4 and other cytokines that downregulate cytotoxic T-cell function), promotion of regulatory T cells (Tregs; and downregulation of T-cell cytotoxic function) and similar mechanisms suggest a plausible and comprehensive hypothesis for how immunomodulation after transfusion is mediated.

Box 13.3 Effects of immunomodulation from allogeneic transfusions demonstrated in animal models

- Enhancement of renal allograft acceptance.
- Increased postoperative infections and mortality from infection.
- Increased metastasis of solid tumours.
- Increased inflammation leading to organ failure and systemic inflammatory response syndrome/multi-organ failure.
- Increased reactivation of latent viral infection.
- Increased lung injury after infusion of DNA, histones and activated platelets in non-leucocyte- reduced donor blood.

Box 13.4 *In vitro* and *in vivo* immunological effects caused by or associated with allogeneic transfusions in model systems, animal models and/or patients

- Reduced responses in mixed lymphocyte culture.
- Reduced cutaneous delayed-type hypersensitivity reactions.
- Reduced responsiveness to mitogens and soluble antigens.
- Decreased natural killer cell number and function.
- Increased CD8 T-cell number and suppressor function.
- Decreased CD4 T-cell number.
- Decreased monocyte–macrophage function.
- Decreased cell-mediated cytotoxicity.
- Increased humoral alloimmunisation to cell-associated and soluble antigens.
- Decreased type 1 (Th1) and increased type 2 (Th2) cytokine secretion.
- Increased T regulatory cell (Treg) numbers and function.
- Neutrophil priming, B-cell, endothelial cell and platelet activation by sCD40L.
- Priming of neutrophil and platelet activation by lipid mediators.
- Generation of free radicals and vascular dysfunction by cell free haemoglobin, haem and iron.
- Vascular dysfunction, platelet activation and a pro-thrombotic state due to nitric oxide scavenging and attendant inflammation.
- Activation of endothelial cells, platelets and innate immunity by microparticles.
- Monocyte and neutrophil recruitment/activation by supernatant cytokines such as interleukin (IL)-6, IL-8, etc.

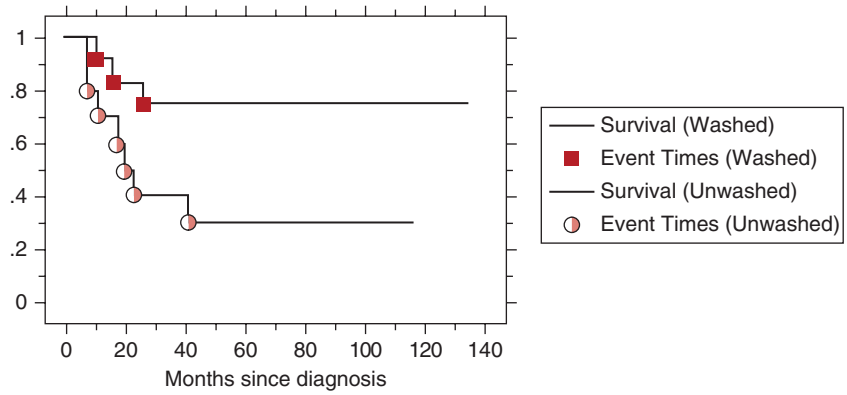

Figure 13.5 Proportion of patients surviving after diagnosis is shown for younger patients (< 50 years of age) with acute leukaemia randomised to washed, leucocyte-reduced, ABO-identical, irradiated red cells and platelets versus unwashed, leucocyte-reduced, ABO-identical irradiated transfusions (n = 12 for washed versus 10 for unwashed); p = 0.03. *Source:* Data from Blumberg et al. [19,20].

Likewise, the effects of non-transferrin-bound iron, free haem and haemoglobin, as documented by the work of Hod, Spitalnik, Gladwin, Triulzi and colleagues (among others), make a coherent and mechanistically credible story as to why red cell transfusions downregulate innate immunity against infection. These transfusion-derived mediators may simultaneously promote thrombosis, inflammation and organ injury, as seen in haemolytic diseases such as sickle cell anaemia and paroxysmal nocturnal haemoglobinuria. The nitric oxide–scavenging effects of stored red cells may contribute to the thromboses, leucocyte, vascular and platelet dysfunction observed in transfused patients. Finally, the plasma-containing supernatant of, in particular, non-leucocyte-reduced red cells, and even of leucocyte-reduced platelets, is rich in many mediators, such as vascular endothelial growth factor (VEGF) and CD40L. Speculatively, these and other mediators may immunomodulate the recipient, contributing to poorer anti-tumour immunity, angiogenesis and inflammation.

These hypothesised mechanisms are likely to operate in concert and may partially explain the associations with transfusion that are proven (renal allograft enhancement and nosocomial infection) and those that may be causal but as yet are not proven (cancer recurrence). It is likely that mechanism will be the last thing that will be clear about transfusion immunomodulation, because not only the administered product(s) but also the clinical settings are variable and complex. Transfusion and its immune effects occur in clinical settings with multiple co-existing diseases, the added effects of anaesthesia, drugs and parenteral fluids. While the mechanism may remain elusive, simple modifications of transfusions, such as leucocyte reduction or washing/stored supernatant removal, may mitigate any devastating clinical outcomes. This is supported by the benefits of leucocyte reduction in surgical patients requiring red cell transfusions, and by the promising results seen with washing/supernatant removal in acute leukaemia (Figure 13.5).

Conclusion

Transfusions alter cellular and innate immunity in recipients. The data are most extensive for red cell transfusions. Favourable effects, such as improved renal allograft survival, are in contrast to adverse effects, such as increased nosocomial infections. Many other associations have been proposed based upon observational and animal model data.

Strategies for mitigating these unfavourable effects that have been demonstrated in randomised trials include leucocyte reduction, use of autologous transfusion, restrictive transfusion criteria and, preliminarily, removal of stored supernatant by washing. Improved techniques of red cell and platelet storage that prevent cellular damage, thus minimising exposure of the recipient to potentially toxic cellular contents such as haemoglobin, microparticles and cytokines, hold promise for further improving the benefit and reducing the risk of blood transfusions.

KEY POINTS

1) The existence of both adverse and beneficial transfusion immunomodulation effects has been convincingly demonstrated for increased postoperative infections and renal allograft enhancement.
2) In cardiac surgery, transfusion of leucocyte-reduced (compared with non-leucocyte-reduced) allogeneic red cells reduces all-cause mortality, likely due to reductions in multi-organ failure.
3) A wide range of adverse and beneficial effects has been demonstrated in transfused patients and animal models that are most likely due to immune effects of allogeneic transfusion, but most of these have not been proven to be causal.
4) A broad range of mechanisms is likely involved, including immune deviation leading to upregulation of humoral immunity and downregulation of cytotoxic cellular immunity, and induction of regulatory T cells.
5) Dysregulation of innate immunity (natural killer cells, macrophages, neutrophils and monocytes) is a prominent component of transfusion immunomodulation. Leucocyte reduction prevents accumulation of leucocytes, platelets, DNA and histones in stored blood components. These mediators may play a role in the effects seen in animal models and patients.
6) Additional manipulations such as washing, improved storage solutions or rejuvenation, re-nitrosylation, avoidance of infusing incompatible ABO antigen and/or antibody, etc. may further reduce the toxicity of blood transfusions by minimising transfusion immunomodulation.

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14 Transfusion-Associated Graft-versus-Host Disease and Microchimerism

Alexandra Budhai[1], *Richard O. Francis*[2] *and Beth H. Shaz*[3]

[1] *Memorial Sloan Kettering Cancer Center, New York, NY, USA*
[2] *Columbia University Medical Center, New York, NY, USA*
[3] *Duke University, Durham, NC, USA*

Transfusion-Associated Graft-versus-Host Disease

Transfusion-associated graft-versus-host disease (TA-GVHD) is an uncommon yet almost always fatal complication of cellular blood component transfusion. TA-GVHD is defined by the UK haemovigilance system Serious Hazards of Transfusion (SHOT) as fever, rash, liver dysfunction, diarrhoea, pancytopenia and bone marrow hypoplasia occurring less than 30 days after transfusion, without other apparent cause. Similarly, the US National Healthcare Safety Network Biovigilance system describes definitive diagnosis as fever, rash, hepatomegaly and diarrhoea between 2 days and 6 weeks after transfusion with laboratory evidence of liver dysfunction, pancytopenia, marrow aplasia, leucocyte chimerism and findings of TA-GVHD on skin or liver biopsy.

Development of TA-GVHD requires the product to contain immunologically competent lymphocytes and the recipient must express tissue antigens absent in the donor and not mount an effective immune response to destroy the foreign lymphocytes. Cellular blood products contain viable lymphocytes that can proliferate and result in TA-GVHD. Inactivation of these lymphocytes, usually through irradiation, prevents TA-GVHD. The identification of individuals at high risk for TA-GVHD, such as immune-impaired patients or those receiving products from blood relatives, and the subsequent requirement that these individuals receive irradiated products reduce the incidence of TA-GVHD. Since the inception of SHOT, 14 cases of TA-GVHD have been reported in the UK. Only 3 of these cases have occurred since the implementation of universal prestorage leuco depletion [1]. No cases have occurred in the UK since 2012; however, the elimination of TA-GVHD could require universal irradiation or lymphocyte inactivation of all cellular blood components.

Pathogenesis

TA-GVHD results from the engraftment of transfused donor T lymphocytes from cellular blood components in a recipient whose immune system does not reject them. Cellular components are defined as red blood cell, platelet and granulocyte components (not frozen plasma). The mechanism of TA-GVHD is like that of acute GVHD after haemopoietic stem cell (HSC) transplantation. Donor T lymphocytes recognise recipient

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human leucocyte antigens (HLAs) as foreign, resulting in activation and proliferation of the lymphocytes, which leads to host cell death and tissue destruction.

Clinical Features

TA-GVHD is an acute illness characterised by fever, rash, pancytopenia, diarrhoea and liver dysfunction, which begins 4–30 days (median 8–10 days) after transfusion and results in death within 3 weeks from symptom onset in over 90% of cases. In neonates, the clinical manifestations are similar, yet the interval between transfusion and onset is longer: the median time of onset of fever is 28 days, rash 30 days and death 51 days [2]. In the typical scenario, fever is the presenting symptom followed by an erythematous maculopapular rash, which begins on the face and trunk and spreads to the extremities. Liver dysfunction manifests as an obstructive jaundice or an acute hepatitis. Gastrointestinal complications include nausea, anorexia or diarrhoea. Leucopenia and pancytopenia, the primary reasons for death due to sepsis, candidiasis and multi-organ failure, develop later and progressively become more severe.

Diagnosis

The diagnosis of TA-GVHD is based on the characteristic clinical manifestations, pathological findings on tissue biopsy and, if possible, evidence of donor-derived lymphocytes in the recipient's blood or affected tissues. Laboratory data demonstrate pancytopenia and abnormal liver function tests. Skin biopsy changes include epidermal basal cell vacuolisation and mononuclear cell infiltration. Liver biopsy findings include degeneration of the small bile ducts, periportal mononuclear infiltrates and cholestasis. The bone marrow is usually hypocellular or aplastic, which is the primary differentiating feature between TA-GVHD and GVHD occurring after HSC transplantation. The discovery of donor lymphocytes or DNA in the patient's peripheral blood or tissue biopsy with the appropriate clinical scenario confirms the diagnosis. Donor-derived DNA is usually detected using polymerase chain reaction (PCR)–based HLA typing; other methods include the use of amplified fragment-length polymorphisms, variable-number tandem repeat analysis, short tandem repeat analysis, microsatellite markers and cytogenetics.

Treatment

Most treatments of TA-GVHD are largely ineffective, but include aggressive use of corticosteroids, antithymocyte globulin, ciclosporin and growth factors. However, spontaneous resolution and successful treatment with a combination of ciclosporin, steroids and OKT3 (anti-CD3 monoclonal antibody) or antithymocyte globulin have been reported. Transient improvement has been seen with nafamostat mesilate, a serine protease inhibitor that inhibits cytotoxic T lymphocytes. There are case reports of successful treatment with autologous or allogeneic HSC transplantation.

Prevention

Since treatment options for TA-GVHD are mostly unsuccessful, patients at increased risk must be identified and transfused with lymphocyte-inactivated products, usually by X-ray or gamma irradiation or pathogen inactivation technologies. Properly installed and maintained gamma irradiation instruments using radioisotopes caesium-137 or cobalt-60 are safe, but their use requires appropriate security, radiation safety protocols and training (e.g. in the USA, blood irradiators are regulated by the Nuclear Regulatory Commission). In addition, these radioisotopes decay over time and take progressively longer to irradiate blood products. Some pathogen inactivation technologies have been shown in human clinical trials, mouse models and other lymphocyte proliferation assays to inactivate T lymphocytes (Table 14.1). In Europe, the use of pathogen inactivation for platelets is growing and gamma irradiation use is

Table 14.1 Potential methods for leucocyte inactivation.

Method	Leucocyte inactivation	Cellular blood components
Gamma irradiation	Inactivates [4]	Cellular blood components
X-rays	Inactivates [4]	Cellular blood components
Amotosalen (S-59) with UVA	Inhibits activation and proliferation	Platelets
	Inhibits TA-GVHD in murine transfusion model [5]	
Riboflavin with UV	Inhibits activation and proliferation [5]	Platelets, whole blood
UVC	Inhibits activation and proliferation [6]	Platelets
S-303 and glutathione	Inactivates	Red blood Cells [5]

TA-GVHD, transfusion-associated graft-versus-host disease; UV, ultraviolet.

decreasing; irradiation by X-ray generated by linear acceleration is increasing in Europe, the USA and other locations as a non-radionuclide source. Due to the security risk of caesium-137 irradiators, in the USA there is a programme in place to decrease the number of caesium irradiators and replace them with X-ray irradiators with government subsidisation. The goal is to completely eradicate caesium irradiators in the USA by 2027. France and Norway have completely replaced their caesium irradiators with X-ray irradiators [3].

Irradiation

Both gamma rays and X-rays inactivate T lymphocytes and can be used to irradiate blood components. Quality assurance measures should be performed for any irradiation technique. For gamma irradiation this includes dose mapping, adjustment of irradiation time to correct for isotopic decay, assurance of no radiation leakage, timer accuracy, turntable operation, preventive maintenance, and a qualitative indicator label to confirm that blood products have been properly irradiated. Verification of dose delivery should occur annually with caesium-137 as a radiation source and semi-annually with cobalt-60 as a radiation source. Quality assurance for X-rays includes dose delivery verification performed according to manufacturer's instructions [4].

The dose of irradiation must be sufficient to inhibit lymphocyte proliferation, but not to significantly damage red cells, platelets and granulocytes or their functions. Assays to assess the effect of irradiation on T-lymphocyte proliferation include mixed lymphocyte culture (MLC) assay and limiting dilution analysis (LDA). The recommended dose varies between 15 and 50 Gy. Of note is that there have been five patients transfused with irradiated blood products, two at doses of 25 Gy, two at doses of 20 Gy, and one at 15 Gy, who developed TA-GVHD [7], but it is unknown whether there was a process or dose failure.

At recommended doses, ionising radiation causes some oxidation and damage to lipid components of membranes, which continue during storage. Products irradiated immediately prior to transfusion appear to be unaffected and have virtually normal function. In stored products, radiation modestly harms red cells, but does not appear to affect platelet and granulocyte function significantly in the clinically utilised doses. The effects on red cells include an increase in extracellular potassium and a decrease in posttransfusion red cell survival. The increase in extracellular potassium is usually not of clinical significance

because of posttransfusion dilution of the potassium. However, there may be certain patients who are sensitive to the increased potassium resulting in transfusion-associated hyperkalaemia, such as fetuses receiving intrauterine transfusions (IUT), premature infants, infants receiving large red cell volumes, and neonates undergoing exchange transfusions or intracardiac transfusions via central line catheters. Irradiating the red cell immediately prior to transfusion can prevent significant potassium increase. Specifically, the UK recommendation is that red cells be transfused within 24 hours for neonates and infants receiving large-volume transfusion [8]. In some scenarios it might be necessary to wash blood products or perform plasma depletion in order to mitigate the amount of potassium the patient receives. Potassium adsorption filters are another potential option to mitigate against for dangerous increases in potassium and are currently in use in Europe. Trials have shown these devices to be both safe and efficacious; however, they have not achieved widespread clinical use [9]. It should be noted that red cell products stored in additive solutions have lower extracellular potassium than CPDA-1 units of a similar age. Due to these changes, red cell product outdate is variably shortened to 14–28 days after irradiation (Table 14.2).

Blood Component Factors

Age of Blood

Use of fresh blood increases the risk of TA-GVHD. A Japanese series of cases of TA-GVHD in immunocompetent patients found that 62% of patients had received blood less than 72 hours old [2]. Based on data from multiple case series, the recent 'Recommendations for the Use of Irradiated Blood Components in Canada' stated that usage of blood stored for more than 14 days may be a potential mitigation strategy for TA-GVHD [10]. The increased risk of fresh blood is possibly due to the function and viability of lymphocytes, as during storage these cells undergo apoptosis and fail to stimulate an MLC response.

Leucocyte Dose

Leucocyte reduction of blood components may decrease the risk of TA-GVHD, but it does not eliminate it. SHOT data reported a substantial decrease in the number of TA-GVHD cases following universal leucocyte reduction of blood components in the UK in 1999 [1].

Blood Components

All cellular blood components, including red cells, platelets, granulocytes, whole blood and fresh plasma (not frozen plasma), contain viable T lymphocytes that are capable of causing TA-GVHD (Table 14.2). Granulocyte transfusions are the highest-risk product because they have a high lymphocyte count and are administered as fresh products to neutropenic and immunosuppressed patients. Therefore, it is recommended that all granulocyte products undergo irradiation prior to transfusion and the remaining cellular blood components be irradiated for patients at increased risk.

Patients at Increased Risk

Patient populations have varying risk factors for developing TA-GVHD (Table 14.3). It is difficult to quantify any of these risks because the number of these patients, the number who are transfused and the number of transfusions or type of products received are unknown. The risk is therefore derived from case reports or haemovigilance data, which are biased by under-recognition, misdiagnosis and under- and passive reporting.

Congenital Immunodeficiency Patients

The first reported cases of TA-GVHD occurred in the 1960s in children with T-lymphocyte congenital immunodeficiency syndromes. Children with severe congenital immunodeficiency syndromes (SCID) and with variable immunodeficiency syndromes, such as Wiskott–Aldrich and DiGeorge syndromes, have developed TA-GVHD. These children may be transfused prior to the recognition of these immunodeficiency syndromes. Because of the possibility of the patient not

Table 14.2 Comparison of irradiation guidelines, including dose and indications.

	UK [8]	USA [4]	Canada [10,11]
Techniques	Gamma irradiation or X-rays	Gamma irradiation or X-rays	Gamma irradiation or X-rays
Dose	Minimum 2500 cGy	2500 cGy at centre of product	2500 cGy at centre of product
	No part > 5000 cGy	Minimum 1500 cGy at any point	Minimum 1500 cGy at any point
		Maximum 5000 cGy	Maximum 5000 cGy
Type of product	All cellular products: Whole blood Red cells Platelets Granulocytes	All cellular products: Whole blood Red cells Platelets Granulocytes	All cellular products: Whole blood Red cells Platelets Granulocytes
Age of product	Red cells – <14 days after collection For hyperkalaemia risk, e.g. exchange or intrauterine transfusion: < 24 h before transfusion	Red cells – any time Platelets – any time	Red cells – < 28 days after collection Platelets – any time
	Platelets – any time		
Expiration	Red cells stored for 14 days after irradiation	Red cells stored for up to 28 days after irradiation or original outdate, whichever is sooner	Red cells stored for 14 days after irradiation and no more than 28 days after collection
General	All blood from relatives	All blood from relatives	All blood from relatives
	All HLA-selected products	All HLA-selected products	All HLA-selected products
	All granulocytes		All granulocytes
Neonates	IUT		IUT
	Exchange transfusions in IUT babies	IUT	All exchange transfusions All transfusions in IUT babies up to 6 months after EDD and LBW babies up to 4 months

Congenital immunodeficiency	All	All	All
Allogeneic HSC transplantation	All – at least 6 months post BMT; longer in selected patients	All	All
Autologous HSC transplantation	All – at least 3 months post BMT; 6 months if TBI used		
Leukaemia	No	*	No
Hodgkin disease	All stages	*	All stages
Purine analogues	All	*	All
Non-Hodgkin lymphoma	Not discussed	*	If receiving purine analogues
Solid tumours	No	*	No
Solid organ transplants	No	*	No
Cardiovascular surgery	No	No	No
AIDS	No	No	No

*According to policies and procedures developed by the blood transfusion laboratory or blood supplier.
AIDS, acquired immune deficiency syndrome; BMT, bone marrow transplant; EDD, estimated date of delivery; HSC, haematopoietic stem cell; HLA, human leucocyte antigen; IUT, intrauterine transfusion; LBW, low birthweight; TBI, total body irradiation.

Table 14.3 Indications for irradiated cellular blood components to prevent transfusion-associated graft-versus-host disease.

Clear indications
Congenital immunodeficiency syndromes (suspected or known)
Allogeneic and autologous haemopoietic progenitor cell transplantation
Transfusions from blood relatives
HLA-matched or partially HLA-matched products (platelet transfusions)
Granulocyte transfusions
Hodgkin disease
Treatment with purine analogue drugs (fludarabine, cladribine and deoxycoformycin)
Treatment of haemotological diagnoses with alemtuzumab (anti-CD52) and other drugs/antibodies that affect T-lymphocyte number or function
Intrauterine transfusions
Indications deemed appropriate by most authorities
Neonatal exchange transfusions
Preterm infants/low-birthweight infants
Infant/child with congenital heart disease (secondary to possible DiGeorge syndrome)
Acute leukaemia
Non-Hodgkin lymphoma and other haematological malignancies
Aplastic anaemia
Solid tumours receiving intensive chemotherapy and/or radiotherapy
Recipient and donor pair from a genetically homogeneous population
Indications unwarranted by most authorities
Solid organ transplantation
Healthy newborns/term infants
HIV/AIDS

AIDS, acquired immune deficiency syndrome; HIV, human immunodeficiency virus; HLA, human leucocyte antigen.

being known to be immunodeficient, it may be prudent to irradiate all blood components for children under a certain age. This is particularly true with infants undergoing cardiac surgery who may have unrecognised DiGeorge syndrome. It is recommended that all patients with suspected or confirmed congenital immunodeficiency receive irradiated products.

Allogeneic and Autologous Haematopoietic Stem Cell Recipients

Both allogeneic and autologous HSC transplant recipients are at increased risk of TA-GVHD. Patients who undergo allogeneic HSC transplantation have received irradiated blood products routinely for over 40 years. Multiple organisations in Europe and the USA recommend irradiated blood products for allogeneic and autologous HSC recipients, but it is unclear for how long before and after transplantation these patients require irradiated blood products.

Leukaemia and Lymphoma Patients

Patients with haematological malignancies are at increased risk for TA-GVHD, especially patients with Hodgkin disease (HD). Twenty cases were reported in patients with malignant lymphoma, 13 in association with HD and 7 with non-Hodgkin lymphoma (NHL), and all undergoing therapy for active disease at the time. Of 14 cases reported to SHOT, 5 were associated with haematological malignancies (Table 14.4). In the 1970s and 1980s,

Table 14.4 Cases of transfusion-associated graft-versus-host disease reported to Serious Hazards of Transfusion (SHOT), 1996–2019.*

Year	Number of cases	Case	Diagnosis and/or possible risk factor	Red cells and/or platelets leuco depletion	Donor-recipient HLA haplotype share
1996–97	4	1	Immunodeficient neonate, not diagnosed at time of transfusion	No	Reported as haplotype share; no other details provided
		2	Epistaxis, age 88	No	NK
		3	B-cell NHL	No	NK
		4	B-cell NHL	No	NK
1997–98	4	5	Waldenstrom's macroglobulinaemia	No	Donor reported as homozygous; no other details provided; patient's HLA type not determined
		6	B-cell NHL	No	Yes; donor homozygous: A1; B8; DR17 Patient: A1, A31; B7, B8, Bw6; Cw7; DR17; DQ2
		7	CABG, red cells less than 3 days old	No	Yes; donor homozygous: A*01; B*0801; DRB1*0301
		8	ITP, treated with prednisolone	No	NK
1998–99	4	9	Myeloma, 6 units of red cells, all less than 7 days old	Yes	NK
		10	Male, age 53; uncharacterised immunodeficiency; HIV negative	No	NK; 100% XX cells in marrow

(*Continued*)

Table 14.4 (Continued)

Year	Number of cases	Case	Diagnosis and/or possible risk factor	Red cells and/or platelets leuco depletion	Donor-recipient HLA haplotype share
		11	CABG, also received platelets	No	NK (32 donors)
		12	CABG	No	Donor homozygous: A* 01; B*0801; Cw*0701/06/07; DRB1*0301; DQB1*0201/02 Patient: A*01, A*3301/03; B*0801, B* 14202/03; Cw*0701/06/07; Cw*0802; DRB1*0301, DRB1*0701/03; DQB1*03032/06, DQB1*0201/02
1999–2000	0				
2000–01	1	13	Relapsed ALL on UKALL R2. Died despite 'rescue' HSC allograft	Yes	NK; chimerism shown by variable-number tandem repeat analysis but no donor HLA typing performed
2012 [12]	1	14	Infant receiving IUT with non-irradiated, non-leuco-depleted maternal blood	No	Parent–child HLA related
Total	**14**				

*No cases were reported from 2002 to 2011 and from 2013 to 2019.
ALL, acute lymphoblastic leukaemia; CABG, coronary artery bypass grafting; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; HSC, haemopoietic stem cell; ITP, immune thrombocytopenia; IUT, intrauterine transfusion; NHL, non-Hodgkin lymphoma; NK, not known.
Source: Williamson et al. 2007 [1]. Reproduced with permission of John Wiley & Sons.

cases of TA-GVHD occurred in patients with acute leukaemia undergoing chemotherapy; the majority of these patients had received granulocyte transfusions. It is recommended that patients with haematological malignancies receive irradiated products; however, it is less clear whether this requirement should be only during active treatment.

Recipients of Fludarabine and Other Purine Analogues as Well as Other Drugs/Antibodies That Affect T-Lymphocyte Number or Function

TA-GVHD was initially reported in patients with chronic lymphocytic leukaemia (CLL) receiving fludarabine, a purine analogue that results in profound lymphopenia. There are nine cases of TA-GVHD in CLL, acute myeloid leukaemia (AML) and NHL patients who received fludarabine up to 11 months prior to transfusion. Other purine analogues, including deoxycoformycin (pentostatin) and chlorodeoxyadenosine (cladribine), have been associated with the development of TA-GVHD. Thus, it is recommended that all patients who have received fludarabine or other purine analogues, as well as alemtuzumab (anti-CD52) or other drugs/antibodies that affect T-lymphocyte function or number, be transfused with irradiated products; however, it is unclear whether this requirement should only be for at least one year and until recovery from the resulting lymphopenia following the administration of these drugs.

Fetus and Neonate

Fetuses and neonates have immature immune systems and may be at increased risk of TA-GVHD. In neonates, most cases of TA-GVHD reported are in those with congenital immunodeficiency or who received products from related donors. At least 10 cases have been reported after neonatal exchange transfusions; 4 occurred in infants who had previously received IUT. Seven cases were in preterm infants (excluding those who received a product from a relative). The use of irradiated products for fetal and neonatal transfusions is recommended for exchange transfusions and IUT, preterm infants, infants with congenital immunodeficiency and those receiving products from relatives; the need for it is less clear for other neonatal transfusions.

Patients with Aplastic Anaemia

Since patients with aplastic anaemia are usually treated with intensive chemotherapy regimens and possible HSC transplantation, some authorities recommend they receive irradiated products, especially during myelosuppressive therapy or treatment with antithymocyte globulin.

Patients Receiving Chemotherapy and Immunotherapy

TA-GVHD has occurred in patients with solid tumours, including neuroblastoma, rhabdomyosarcoma and bladder and small cell lung cancer, during intensive myeloablative therapy. Therefore, it is recommended that patients with solid tumours receive irradiated products during myelosuppressive therapy.

Solid Organ Transplantation Recipients

GVHD is a rare complication of solid organ transplantation, which usually results from the passenger lymphocytes contained within the solid organ and not from transfusion, even though these individuals are highly immunosuppressed and transfused. There have been five cases of TA-GVHD in recipients of solid organs including liver and heart, and inconclusive cases in kidney transplant recipients. The risk of TA-GVHD in solid organ transplant recipients appears to be low and the use of irradiated products is generally considered to be unwarranted.

Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) patients

HIV/AIDS is not considered a risk factor for TA-GVHD, as there is only a single case report of a child with AIDS developing transient TA-GVHD. The use of irradiated blood products in HIV/AIDS patients is not warranted.

Immunocompetent Patients

TA-GVHD has been reported in immunocompetent patients, especially those who received transfusions of blood products donated by close relatives [13], most likely leading to a one-way haplotype match in which the donor was homozygous for a haplotype for which the recipient was heterozygous, allowing donor lymphocytes to evade immune detection, yet still respond to donor tissue. The risk of receiving a blood product from a homozygous donor is greatest in populations with limited HLA haplotype polymorphisms, such as Japan (Table 14.5). A systematic review of 348 TA-GVHD cases found that most of the cases involved transfusions in immunocompetent patients, with cellular blood components stored for ≤ 10 days, and a donor HLA profile lacking identifiably foreign antigen types compared to the recipient [14], demonstrating the importance of viable lymphocytes that are able to evade immune detection in causing TA-GVHD. Irradiation of products from close relatives and HLA-matched products are recommended, but the risk is minimal for other immunocompetent patients.

Guidelines and Requirements for Irradiated Products

The American Society for Clinical Pathology [15] and the British Society for Haematology in the UK [8] published guidelines for the use of irradiation to prevent TA-GVHD. Japan has elected to irradiate all blood products [5]. In 2014, College of American Pathologists (CAP) members were surveyed about their blood product irradiation practices [16]. The most frequent indications for blood product irradiation were transfusion from blood relatives (78.6%), HLA-matched or partially matched products (68.9%), neonatal exchange transfusions (66.3%), IUT (63.3%), HSC transplant (62.7%) and preterm/low-birthweight infants (61.8%). While these patients are generally considered at risk for TA-GVHD, it was noted that several patient populations not considered at risk, including patients with HIV/AIDS (19.5%) and autoimmune disorders (17.5%), are included in some organisations' irradiation policies. These results suggest that irradiation practices remain heterogeneous and that continued efforts to standardise irradiation practices are warranted.

Universal Irradiation

As case reports cited above indicate, TA-GVHD can occur in immunocompetent patients and individuals where the degree of immunocompromise was not known or properly identified prior to transfusion. Given that TA-GVHD is fatal in almost all cases and the risk of radiation of a product includes only minimal cost and effect on

Table 14.5 Frequency of homozygous HLA donors in various populations.

Frequency of transfusion from homozygous donors to potential heterozygous recipients			
Population	**Parent/child**	**Sibling**	**Unrelated**
Japan	1 : 102	1 : 193	1 : 874
Canada Whites	1 : 154	1 : 294	1 : 1664
Germany	1 : 220	1 : 424	1 : 3144
Korea	1 : 183	1 : 356	1 : 3220
Spain	1 : 226	1 : 438	1 : 3552
South Africa Blacks	1 : 286	1 : 558	1 : 5519
USA Whites	1 : 475	1 : 920	1 : 7174
Italy	1 : 434	1 : 854	1 : 12 870
France	1 : 762	1 : 2685	1 : 16 835

product potency, many authorities are in favour of universal irradiation. In 2000 in Japan, the use of irradiated cellular blood products became widespread, and no cases of TA-GVHD have been confirmed since that time [5]. If TA-GVHD cases were to persist in immunocompetent patients, or in populations where products are not typically irradiated (e.g. solid organ transplant recipients), consideration of universal irradiation could be undertaken as appropriate.

Pathogen Inactivation

Pathogen inactivation (PI) technologies are not used in the UK for the prevention of TA-GVHD [8]; however, they may be used in the USA and other countries as long as the technology has been shown to inactivate residual leucocytes. When comparing PI technology to irradiation, an *in vitro* study showed that PI by amotosalen and ultraviolet (UV) A exhibited no T-cell proliferation detected after 1×10^7 peripheral blood mononuclear cells (PBMCs) were cultured. Gamma irradiation was only able to completely inactivate T cells when 1×10^5 PBMCs were cultured [17]. Data regarding the routine use of amotosalen and UVA for platelet units show that no instances of TA-GVHD have been noted out of 2 million platelet transfusions reported to the manufacturer. In contrast, TA-GVHD has been seen with irradiation when it has been underdosed, and even at a dose of 25 Gy [9]. Whether PI technology for the prevention of TA-GVHD will be widely adopted remains to be seen.

Haemovigilance

Many countries have comprehensive tracking systems for adverse events of blood transfusion (see Chapter 21). The continued occurrence of TA-GVHD is likely to be because of lack of agreement on the level of immunodeficiency that results in increased risk and patients with immunocompromised conditions who receive non-irradiated products, either secondary to not being identified prior to transfusion or the product not being irradiated by error. SHOT data state that there were 1478 cumulative patients with missed irradiation between 1999 and 2018, with events occurring in 81 patients in 2018. One missed irradiation event resulted in TA-GVHD in 2012 [13]. Two more UK cases of TA-GVHD resulted from scenarios where the immunodeficient status of the patients was not known until after they were transfused [1]. US transfusion services reported 127 missed irradiation events in 2018 and again in 2019, but no TA-GVHD fatality has been reported to the FDA since 2011 [18]. The low incidence of reported TA-GVHD may be secondary to under-reporting and/or under-recognition, the fact that lymphocytes are no longer capable of proliferating because the blood is older by the time of transfusion, or decreasing risk due to leucocyte reduction of blood products and the genetic heterogeneity of many populations.

Transfusion-Associated Microchimerism

Chimerism is defined as the presence of two genetically distinct cell lines in a single organism. Haemopoietic chimerism refers to the persistence of allogeneic donor lymphocytes in a recipient. Microchimerism (MC) occurs when these donor cells represent a small population (less than 5%) and can be a consequence of pregnancy, organ transplantation or transfusion.

Clinical Data

Transfusion-associated microchimerism (TA-MC) has been reported mostly in trauma patients. It has also been reported in sickle cell disease and thalassemia patients, and in a recent study of paediatric patients with anaemia in Ghana, contradicting the idea that MC is unlikely to be found in a non-trauma setting. TA-MC can be sustained for decades after transfusion. It is unknown whether it represents long-term survival of lymphocytes or haematopoietic engraftment in the transfusion recipient.

Irradiation of products prevents TA-MC; however, there are mixed data concerning whether leucocyte reduction alone can decrease the incidence of TA-MC among trauma patients. One recent study has found a lower proportion of short- and long-term MC than described in prior research, which may be attributed to updates in leucocyte reduction methods and other changes in blood product processing that have produced modest decreases in residual leucocytes [19]. Leucocyte reduction may be protective of TA-MC in non-trauma patients, as shown in a study of TA-MC in patients undergoing cancer surgery [20]. The clinical sequelae of TA-MC are unknown. One study reported a decrease in donor-specific lymphocyte response in TA-MC trauma patients versus non-TA-MC patients [19]. TA-MC has also been associated with immunomodulatory effects such as increased IL-10 production in comparison to non-TA-MC patients [20]. To date, no clear relationship of TA-MC to clinical outcomes has been elucidated.

KEY POINTS

1) Transfusion-associated graft-versus-host disease (TA-GVHD) is a rare yet largely fatal complication of cellular blood component transfusion.
2) TA-GVHD can be prevented by using irradiated or leucocyte-inactivated blood components.
3) Patients at increased risk for TA-GVHD include those who are immune impaired and those receiving blood components donated from blood relatives.
4) Leucocyte dose and age of the blood component, HLA matching between the donor and the recipient and immune state of the recipient contribute to the likelihood of developing TA-GVHD.
5) While there are strong data for providing irradiated blood components to prevent TA-GVHD in some patient populations, the need for irradiation in other disease states is less clear.
6) Microchimerism can be detected after transfusion, but the conditions that facilitate it and its clinical consequences are unknown.

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15 Posttransfusion Purpura

Michael F. Murphy

Radcliffe Department of Medicine, University of Oxford; NHS Blood and Transplant, John Radcliffe Hospital; Department of Haematology, Oxford University Hospitals, Oxford, UK

In 1959, van Loghem and colleagues described a 51-year-old woman who developed severe thrombocytopenia seven days after elective surgery [1]. The thrombocytopenia did not respond to transfusion of fresh blood, but there was a spontaneous recovery after three weeks. The patient's serum contained a strong platelet alloantibody, which enabled the description of the first human platelet antigen (HPA; Zw, see Chapter 5). However, the relationship of platelet alloimmunisation to posttransfusion thrombocytopenia was not recognised until two years later when Shulman and colleagues studied a similar case, naming the antibody anti-Pl^{A1} (later shown to be the same as anti-Zw) and coined the term posttransfusion purpura (PTP) [2].

Definition

PTP is an acute episode of severe thrombocytopenia occurring about a week after a blood transfusion. It usually affects HPA-1a-negative women who have previously been alloimmunised by pregnancy. The transfusion precipitating PTP causes a secondary immune response, boosting the HPA-1a antibodies, although the mechanism of destruction of the patient's own HPA-1a-negative platelets remains uncertain.

Incidence

PTP is considered to be a rare complication of transfusion. Over 200 cases had been reported in the literature up to 1991 [3]. However, this may not reflect the true incidence of PTP, which is not known except through reporting to haemovigilance schemes. In the first four years of the Serious Hazards of Transfusion (SHOT) scheme during which approximately 13 million blood components were transfused, 37 cases were reported, giving an approximate incidence of 1 case in 350 000 transfusions. In the following 13 years, after the introduction of universal leucocyte reduction of blood components in the UK, only 19 cases were reported to SHOT, giving an approximate incidence of 1 in 2 million blood components transfused [4]. Only one case of probable PTP has been reported to SHOT in the last three years [5]. A study of elderly patients (65 years and older) in the USA found an incidence of PTP of 1.8 in 100 000 inpatient admissions [6]. Significantly higher odds of PTP were found with platelet-containing transfusion events and with a greater number of units transfused.

The low incidence of PTP relative to the 2.1% of the population who are HPA-1a negative and at risk of the condition raises the

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question of individual susceptibility. As in neonatal alloimmune thrombocytopenia (NAIT), the antibody response to HPA-1a is strongly associated with a certain human leucocyte antigen (HLA) class II type (HLA-DRB3*0101) (see Chapter 5).

Clinical Features

PTP typically occurs in middle-aged or elderly women (mean 57 years, range 21–80), although it has also been found in males [6,7]. All patients, apart from rare exceptions, have had previous exposure to platelet antigens through pregnancy and/or transfusion. The interval between pregnancy and/or transfusion and PTP is variable, the shortest being 3 years and the longest 52 years. The initial maternal sensitisation to platelet antigens during pregnancy in females subsequently developing PTP is rarely of sufficient degree to cause NAIT.

Blood components implicated in causing PTP are:

- Whole blood.
- Packed red cells.
- Red cell concentrates.
- Platelet concentrates.

There are occasional case reports of PTP following the transfusion of plasma, presumably due to the presence of platelet particles expressing platelet antigens [7].

Severe thrombocytopenia and bleeding usually occur about 5–12 days after transfusion; shorter or longer intervals are rare. The onset is usually rapid, with the platelet count falling from normal to $< 10 \times 10^9$/L within 12–24 hours. Haemorrhage is very common and sometimes severe. There is typically widespread purpura and bleeding from mucous membranes and the gastrointestinal and urinary tracts. In many cases the precipitating transfusion has been associated with a febrile non-haemolytic transfusion reaction, probably due to the presence of co-existing HLA antibodies stimulated by previous pregnancy and/or transfusion.

Megakaryocytes are present in normal or increased numbers in the bone marrow and coagulation screening tests are normal in uncomplicated PTP.

In untreated cases the thrombocytopenia usually lasts between 7 and 28 days, although it occasionally persists for longer.

Differential Diagnosis

The rapid onset of severe thrombocytopenia in a middle-aged or elderly woman should arouse suspicion of PTP and a history of recent blood transfusion should be sought. The differential diagnosis includes other causes of acute immune thrombocytopenia such as the following:

- Autoimmune thrombocytopenia.
- Drug-induced thrombocytopenia, e.g. heparin-induced thrombocytopenia (HIT; see Chapter 16).
- Non-immune platelet consumption, e.g. disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura (TTP).
- A less likely possibility is passively transfused platelet-specific alloantibodies from an immunised blood donor when thrombocytopenia occurs within the first 48 hours after the transfusion [8,9].
- Pseudothrombocytopenia due to ethylenediamine tetra-acetic acid (EDTA)–dependent antibodies should be excluded in any patient with unexplained thrombocytopenia by examination of the blood film.

Laboratory Investigations

A preliminary diagnosis of PTP on clinical grounds needs to be confirmed by the detection of platelet-specific alloantibodies. The majority (80–90%) of cases of PTP are associated with the development of HPA-1a antibodies in HPA-1a-negative patients [7,10]. Antibodies against HPA-1b, HPA-2b, HPA-3a, HPA-3b, HPA-4a, HPA-5a, HPA-5b, HPA-15b and Nak[a] (CD36) have also been

associated with PTP, and combinations of antibodies may be present, e.g. anti-HPA-1a and anti-HPA-5b.

HLA antibodies are often present in patients with PTP. There is no evidence that they are involved in causing PTP, but their presence complicates the detection of platelet-specific antibodies. Modern platelet serological techniques such as the monoclonal antibody immobilisation of platelet antigens (MAIPA) assay are useful for resolving mixtures of antibodies in patients with PTP (see Chapter 5).

Pathophysiology

The typical time course of events in PTP is shown in Figure 15.1. A blood transfusion triggers a rapid secondary antibody response against HPA-1a, and there is acute thrombocytopenia about a week after the transfusion. It is difficult to understand why the patient's own HPA-1a-negative platelets are destroyed. There remains no generally accepted mechanism to explain this, although a number of suggestions have been made as follows:

- Transfused HPA-1a-positive platelets release HPA-1a antigen, which is adsorbed onto the patient's HPA-1a-negative platelets, making them a target for anti-HPA-1a. Support for this hypothesis comes from observations such as the elution of anti-HPA-1a from HPA-1a-negative platelets in some cases of PTP, and the demonstration of the adsorption of HPA-1a antigen onto HPA-1a-negative platelets after incubation with plasma from HPA-1a-positive stored blood [11].
- The released HPA-1a antigen forms immune complexes with anti-HPA-1a in the plasma, and the immune complexes become bound to the patient's platelets, causing their destruction.
- The transfusion stimulates the production of platelet autoantibodies as well as anti-HPA-1a. Evidence in favour of this mechanism is the detection of positive reactions of some PTP patients' sera from the acute thrombocytopenic phase with autologous platelets.
- In the early phase of the secondary antibody response, anti-HPA-1a may be produced, which has the ability to cross-react with autologous as well as allogeneic platelets.

Management

Immediate treatment is essential, as the risk of fatal haemorrhage is greatest early in the course of PTP. In a review of 71 cases of PTP, 5 died within the first 10 days because of

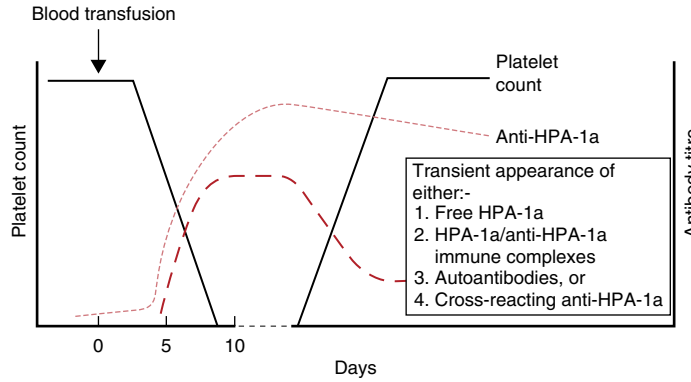

Figure 15.1 A typical time course of posttransfusion purpura. Purpura and severe thrombocytopenia occurred 5–10 days after a blood transfusion. The figure indicates the secondary antibody response of anti-HPA-1a, and the postulated transient appearance of free HPA-1a antigen in the plasma, which binds to HPA-1a-negative platelets, HPA-1a/anti-HPA-1a immune complexes, platelet autoantibodies or cross-reacting HPA-1a antibodies.

intracranial haemorrhage [7]. The main aim of treatment is to prevent severe haemorrhage by shortening the duration of severe thrombocytopenia.

No randomised controlled trials of treatment for PTP have been carried out. Comparison of various therapeutic measures is complicated, because it may be difficult to differentiate a response to treatment from a spontaneous remission in individual cases.

High-dose intravenous immunoglobulin (IvIgG; 1–2 g/kg given over 2 or 5 days) is the current treatment of choice, with responses in about 80% of cases [12]; there is often a rapid increase in the platelet count within 48–72 hours (Figure 15.2) [13]. Steroids and plasma exchange were the preferred treatments before the availability of IVIgG, and plasma exchange, in particular, appeared to be effective in some but not all cases [7].

Platelet transfusions are usually ineffective in raising the platelet count, but may be needed in large doses to control severe bleeding in the acute phase, particularly in patients who have recently undergone surgery before there has been a response to high-dose IvIgG. There is no evidence that

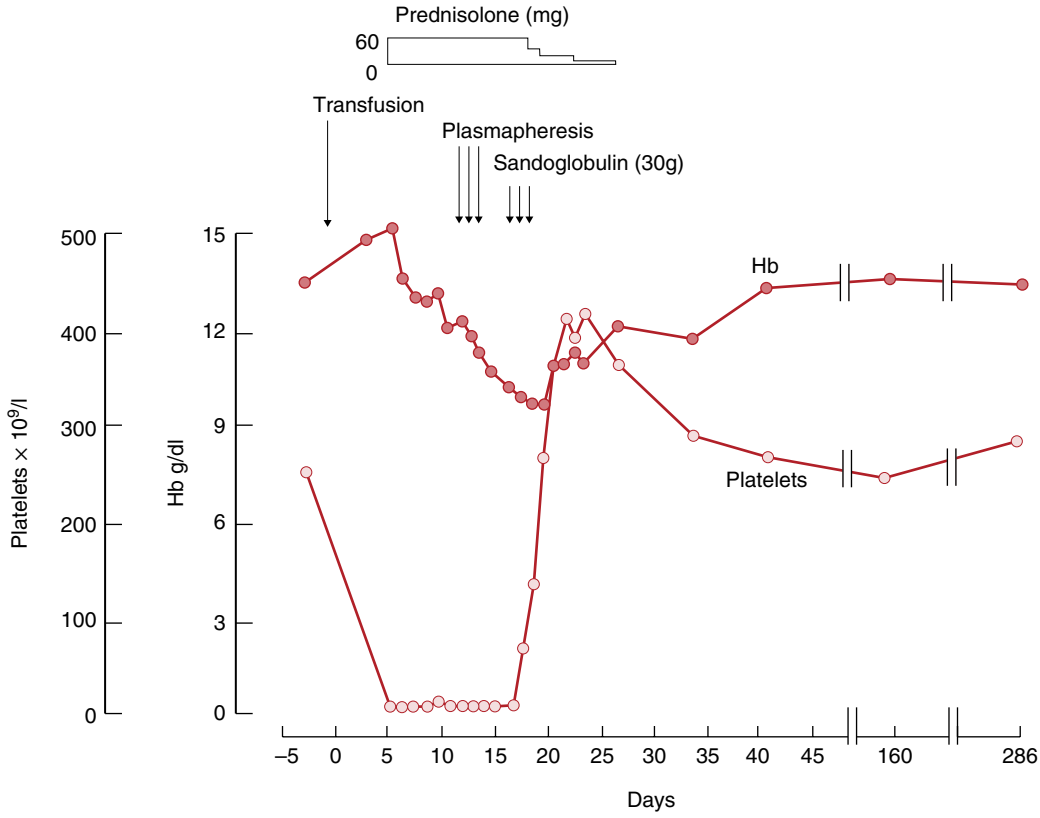

Figure 15.2 Haematological course of a patient with posttransfusion purpura showing the onset of profound thrombocytopenia 6 days after a blood transfusion. Initial treatment with random platelet concentrates caused rigors and bronchospasm, and there was no platelet increment. There was no response to prednisolone (60 mg/day) or plasma exchange (2.5 L/day for 3 days), but there was a prompt remission following high-dose IvIgG (30 g/day for 3 days). *Source*: Adapted from Berney et al. 1985 [13].

platelet concentrates from HPA-1a-negative platelets are more effective than those from random donors in the acute thrombocytopenic phase. There is also no evidence to suggest that further transfusions in the acute phase prolong the duration or severity of thrombocytopenia.

Platelet transfusions have been reported to cause severe febrile and occasionally pulmonary reactions in patients with PTP; these were probably due to HLA antibodies reacting against leucocytes in non-leucocyte-reduced platelet concentrates.

Prevention of Recurrence of Posttransfusion Purpura

Recurrence of PTP has been reported. However, it is unpredictable and has usually occurred following a delay of three years or more after the first episode. The patient should be issued with a card to indicate that they have previously had PTP and 'special' blood is required for future transfusions.

Future transfusion policy should be to use red cell and platelet concentrates from HPA-compatible donors or autologous transfusion.

KEY POINTS

1) Posttransfusion purpura (PTP) is characterised by an acute episode of severe thrombocytopenia occurring about a week after a transfusion.
2) The pathophysiology remains uncertain.
3) PTP typically occurs in HPA-1a-negative women who have been alloimmunised by pregnancy.
4) Haemorrhage is common and sometimes severe, although the thrombocytopenia resolves spontaneously within a few weeks.
5) High-dose intravenous immunoglobulin (IvIgG) (2 g/kg given over 2 or 5 days) is the current treatment of choice to shorten the duration of thrombocytopenia, with responses in about 80% of cases.
6) Universal leucocyte reduction of blood components in the UK has resulted in a marked reduction in the number of reported cases.

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16 Heparin-Induced Thrombocytopenia

Theodore E. Warkentin[1] *and Andreas Greinacher*[2]

[1] *Department of Pathology and Molecular Medicine and Department of Medicine, Michael G. DeGroote School of Medicine, McMaster University; McMaster Centre for Transfusion Research; Transfusion Medicine, Hamilton Regional Laboratory Medicine Program and Service of Benign Hematology, Hamilton Health Sciences, Hamilton General Hospital, Hamilton, Ontario, Canada*
[2] *Department of Immunology and Transfusion Medicine, Universitätsmedizin Greifswald, Greifswald, Germany*

Heparin-induced thrombocytopenia (HIT) is an antibody-mediated adverse effect of heparin. It is highly prothrombotic and treatment usually requires substitution of heparin with a rapidly acting non-heparin anticoagulant; vitamin K antagonists (warfarin) are contraindicated during the acute phase of HIT because their use can precipitate limb necrosis due to microthrombosis associated with depletion of protein C (vitamin K-dependent natural anticoagulant). Prophylactic platelet transfusions should be minimised. An emerging treatment in addition to alternative anticoagulation in severe HIT is high-dose intravenous immunoglobulin (IvIg), which abruptly inhibits HIT antibody-induced platelet activation. Given the special treatment aspects of HIT, the challenge is to distinguish HIT from non-HIT thrombocytopenia. Management of HIT requires knowledge of immunohaematology and haemostasis. Table 16.1 lists the features of HIT of relevance for the transfusion medicine specialist. At the end of this chapter, we also discuss a HIT-mimicking disorder, vaccine-induced immune thrombotic thrombocytopenia (VITT).

Pathogenesis

Figure 16.1 illustrates the pathogenesis of HIT. Key features include the following:

- Antigens form when platelet factor 4 (PF4) – a positively charged 31 kDa tetrameric member of the C-X-C subfamily of chemokines – forms multimolecular complexes with negatively charged heparin when both are present at stoichiometrically optimal concentrations (1 : 1 to 2 : 1 ratio of PF4 : heparin) [1].
- Both PF4 and heparin bind to platelet surfaces; thus, *in situ* formation of PF4/heparin complexes on platelet membranes localises subsequent formation of PF4/heparin/IgG immune complexes also to the platelet surfaces, i.e. there are no circulating immune complexes in HIT.
- 'Natural' IgM recognises PF4/heparin complexes, leading to activation of complement by the classical pathway; complement helps to stabilise large immunogenic PF4/heparin complexes, and also helps localise PF4/heparin/IgG complexes onto cell surfaces [2]. This enhances recognition

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Table 16.1 Heparin-induced thrombocytopenia (HIT) issues relevant to transfusion medicine.

HIT-related item	Transfusion medicine-related comment
PF4/heparin complexes form at optimal stoichiometric ratio	The Coombs' test requires an optimal concentration of the antihuman immunoglobulin antibody to achieve agglutination of red cells
Acute HIT activates platelets, monocytes, endothelial cells and the coagulation cascade	Acute haemolytic transfusion reaction activates platelets, leucocytes, endothelial cells and the clotting cascade
Typical-onset HIT (day 5–14)	Timing resembles that of delayed haemolytic transfusion reaction in preimmunised patients
Rapid-onset HIT (< 1 day)	Timing resembles that of acute haemolytic transfusion reaction (i.e. due to pre-existing anti-red-cell alloantibodies)
'Delayed-onset' HIT antibodies bind to and activate platelets even in the absence of heparin (platelet-derived polyphosphates and chondroitin sulfate substitute for heparin)	In post-transfusion purpura, alloantibodies boosted by transfusing HPA-1a-positive platelets bind to the patient's own (HPA-1a-negative) platelets, causing severe thrombocytopenia
Functional (platelet activation) assays are more predictive for HIT than immunoassays	HLA antibodies that test positive in lymphocytotoxicity tests are more clinically relevant compared to ELISA-only reactive HLA antibodies
Most ELISA-positive patients do not develop HIT	Many individuals with a positive direct antihuman globulin (Coombs') test do not have immune haemolytic anaemia
Particle gel immunoassay	Rapid assay utilising gel card technology commonly used in transfusion medicine
Platelet transfusions (prophylactic)	Relatively contraindicated in HIT
PCCs contain heparin	PCCs are relatively contraindicated during acute HIT
High-dose intravenous IgG (IvIg)	IvIg appears to be effective as adjunctive treatment for severe HIT, especially in autoimmune HIT (persisting, spontaneous, etc.)

ELISA, enzyme-linked immunosorbent assay; HLA, human leucocyte antigen; PCC, prothrombin complex concentrates; PF4, platelet factor 4.

of PF4/heparin complexes by specific B cells and so induction of anti-PF4/heparin IgG antibodies.

- The HIT antigen(s) reside(s) on PF4, rather than on heparin; indeed, non-heparin polyanions (e.g. polyvinyl sulfonate [PVS] or RNA- or DNA-based drugs) can substitute for heparin in forming HIT antigens.
- Ultra-large PF4/heparin complexes are more readily formed with unfractionated heparin (UFH) than with low molecular weight heparin (LMWH), perhaps explaining the 10-fold greater risk of HIT with UFH versus LMWH.
- Heparin causes platelet activation and release of PF4. However, immunisation occurs most often post surgery and in patients with major trauma (perhaps reflecting a cooperative effect of greater PF4 release from activated platelets and pro-inflammatory factors).
- Anti-PF4/heparin antibodies become detectable around four days (median) after an immunising heparin exposure, with detection of platelet-activating antibodies one or two days later [3,4].
- Anti-PF4/heparin antibodies of IgG and/or IgA and/or IgM can be formed (relative

frequency IgG > IgA > IgM). However, only IgG antibodies have the potential to cause HIT, because platelet activation occurs only when multimolecular complexes of PF4/heparin/IgG result in clustering of the platelet Fc receptors (FcγIIa), causing intravascular platelet activation.

- HIT does *not* exhibit features of a classic primary immune response. Even when HIT occurs during a patient's very first exposure to heparin, IgG antibodies are readily detected after only four to five days, whereas IgM antibodies usually are not detected. If IgM antibodies are found, they become detectable at the same time as IgG.
- These atypical features of the HIT immune response could reflect pre-sensitisation due to exposure to bacteria, as negatively charged molecules on bacterial surfaces bind PF4 in a way that exposes HIT antigens [5].
- Platelet activation in HIT includes formation of procoagulant platelet-derived microparticles.
- Other procoagulant features of HIT include 'pancellular' activation, including expression of tissue factor by activated monocytes and endothelial cells, release of neutrophil extracellular traps ('netosis') [6] and neutralisation of heparin by PF4.
- HIT shows features of innate immunity (involvement of complement, natural IgM, granulocytes and netosis) as well as adaptive immunity (IgG, Fc receptor-dependent activation of monocytes). Combined, uncontrolled activation of both pathways may explain the catastrophic outcomes in severely affected patients.
- Sometimes, HIT resembles an autoimmune disorder. So-called autoimmune (aHIT) antibodies strongly activate platelets in the absence of pharmacological heparin (heparin-'independent' platelet activation); resulting aHIT disorders include 'delayed-onset' HIT [7], 'persisting (refractory) HIT' [8] and 'spontaneous HIT syndrome' [9] (see below).

Epidemiology

- The overall frequency of HIT among heparin-exposed inpatients is ~0.2%.
- The frequency of HIT approaches 5–10% when there are multiple concurrent risk factors for HIT, for example (a) UFH use (versus LMWH or fondaparinux) for (b) at least 10–14 days (when antibodies peak), (c) post orthopaedic surgery and (d) female sex (1.5–2.0 × greater risk of HIT in females versus males).
- HIT occurs more often in post-surgery patients than in medical patients. HIT is rare in pregnancy and in paediatric patients, and probably does not occur in neonates.
- UFH is rarely administered nowadays to post-orthopaedic surgery patients. Thus, HIT occurs most often in post-cardiac/post-vascular surgery patients and general surgery patients who receive postoperative UFH thromboprophylaxis.
- Rarely, a transient HIT-mimicking syndrome with thrombocytopenia, thrombosis and high levels of platelet-activating anti-PF4/heparin antibodies can occur without proximate exposure to heparin, but after infection or surgery ('spontaneous HIT') [9].

Heparin-Induced Thrombocytopenia: A 'Clinicopathological' Syndrome

Table 16.2 summarises the major clinical and laboratory features of HIT.

Iceberg Model

See Figure 16.2.

- HIT occurs in a minority of patients who form anti-PF4/heparin antibodies; anti-PF4/heparin antibodies are detectable in 50–80% of post-cardiac surgery patients, yet HIT occurs in only 1–2% of these patients.
- According to the 'iceberg model', HIT occurs in the subset of patients who form strong heparin-dependent, platelet-activating antibodies of the IgG class that are readily detectable by PF4-dependent enzyme-linked immunosorbent assay (ELISA) [10].

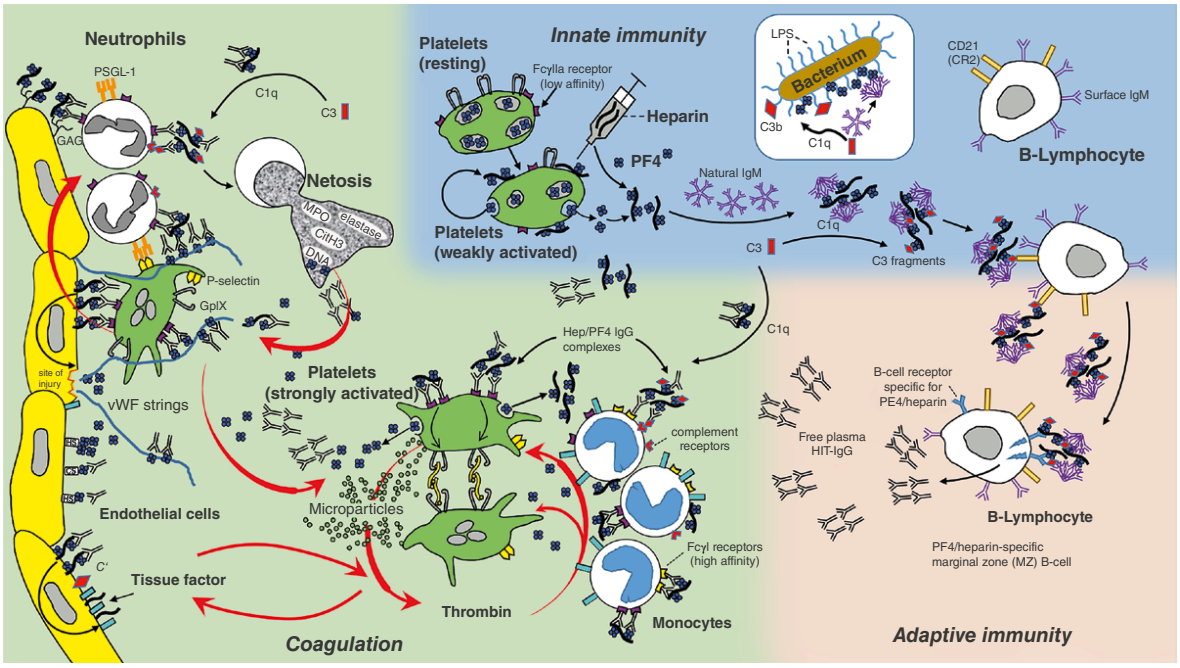


Figure 16.1 Heparin-induced thrombocytopenia (HIT) pathogenesis bridges innate immunity, adaptive immunity and coagulation.

Innate immunity (blue section in upper-right portion of the figure): HIT is a misdirected bacterial host defence. As shown in the white inset, a bacterium – with (negatively charged) surface lipopolysaccharide (LPS) – binds (positively charged) platelet factor 4 (PF4), and conformational changes within PF4 expose neoepitope(s) recognised by 'natural' immunoglobulin M (IgM). Natural IgM binds complement factor 3 (C3), which together with C1q converts C3 to C3 fragments (C3 products), such as C3b, which binds to bacterial surfaces, thus facilitating opsonisation of bacteria (not shown).

In heparin-treated patients, circulating PF4 arises from two sources. Heparin directly binds to platelets, resulting in 'weak' platelet activation that nonetheless is sufficient to result in release of some PF4 from platelet α-granules. In addition, heparin leads to displacement of PF4 from endothelial cell heparan sulphate (HS; shown on the endothelial surface in the bottom-left section of the figure).

Adaptive immunity (pink section in bottom-right portion of the figure): In a subset of B lymphocytes that express B-cell receptors (i.e. surface immunoglobulin) specific for PF4/heparin complexes, the multimolecular PF4/heparin complexes cross-link the B-cell receptors, thereby inducing activation of those B lymphocytes, leading ultimately to formation of plasma cells capable of producing anti-PF4/heparin antibodies, including immunoglobulin G (HIT-IgG) of widely varying pathogenicity – ranging from clinically irrelevant anti-PF4/heparin antibodies to strongly platelet-activating antibodies. Marginal zone (MZ) B lymphocytes are mainly involved in the anti-PF4/heparin immune response; the role of T lymphocytes remains debated.

Coagulation (HIT-associated hypercoagulability) (green section in left-most portion of the figure): Anti-PF4/heparin antibodies of IgG class can bind to PF4/heparin complexes, leading to formation of ultra-large PF4/heparin/HIT-IgG immune complexes. These complexes are assembled primarily on cell surfaces. Indeed, free plasma IgG appears to be required in order to allow for assembly of cell surface PF4/heparin/IgG immune complexes. Target cells to which PF4/heparin/IgG complexes can bind include platelets, monocytes, endothelium and neutrophils.

Platelets bear (low-affinity) FcγIIa receptors and, despite their low affinity, the tropism of heparin for platelet surfaces means that PF4/heparin/IgG complexes form on platelet surfaces, with the Fc 'tails' binding to the platelet FcγIIa receptors, leading to a 'strong' platelet-activation response, so inducing thrombocytopenia and formation of platelet-derived microparticles, which provide the catalytic surface for thrombin generation.

In addition, PF4/heparin/HIT-IgG immune complexes also form on monocyte surfaces, resulting in binding to and complexing of both high-avidity (FcγI) and low-avidity (FcγIIa) Fc receptors to cause cellular activation. Activated monocytes express tissue factor, further contributing to thrombin generation. Activated platelets also bind to endothelial cells (which however do not bear Fcγ receptors). Nonetheless, anti-PF4/heparin antibodies along with platelets appear to activate endothelial cells, which may then express tissue factor so contributing to thrombin generation.

Endothelial cells can also express large multimers of von Willebrand factor (vWF). PF4 binds to vWF strings, undergoing the same conformational change as occurs with binding to other polyanions. Consequently, anti-PF4/heparin antibodies bind to vWF-bound PF4, as do platelets (which bind to vWF via their GP IbIX receptors), leading to platelet activation (particularly at sites of vascular injury where vWF strings are formed).

Neutrophils also bind PF4/heparin/HIT-IgG complexes via FcγIIa receptors; see upper-left panel), leading to neutrophil activation, which results in binding to activated endothelial cells and to vWF. Neutrophils also interact with platelets in HIT, via P-selectin (on platelet surfaces) binding to P-selectin glycoprotein ligand-1 (PSGL-1) on neutrophil surfaces. Activation of neutrophils in HIT results in neutrophil 'netosis', i.e. release of neutrophil extracellular traps (NETs), which contain DNA, citrullinated histone H3 (CitH3), myeloperoxidase (MPO) and elastase. The released DNA of the NETs binds PF4 and vWF, which expresses new binding sites for anti-PF4/heparin antibodies and further formation of HIT immune complexes. The feed-forward loops further promote thrombin generation.

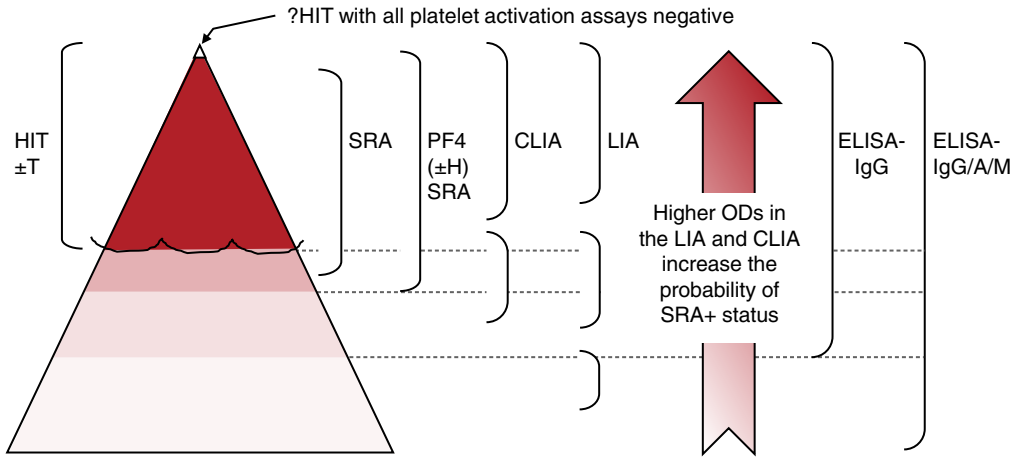
The extreme pathogenicity of HIT – one of the most prothrombotic disorders in medicine – likely reflects this complex activation of longstanding evolutionary mechanisms aimed originally at protecting the host of negatively charged invaders such as bacteria. The strong negative charge of heparin (and certain other polyanions) induces this cascade of host defence mechanisms, as complexes of negatively charged molecules mimic the 'danger signal' of microbial invasion.

The key treatment approaches in HIT are inhibition of thrombin generation (anticoagulation) and interruption of the Fcγ receptor-mediated cell signalling (high-dose IvIg treatment) involving platelets, monocytes and neutrophils, and their self-enhancing prothrombotic mechanisms.

Table 16.2 Heparin-induced thrombocytopenia (HIT) viewed as a clinical–pathological syndrome.

Clinical	Pathological
One or more of: • **Thrombocytopenia** • **Thrombosis** (e.g. *venous:* DVT, pulmonary embolism, venous limb gangrene, cerebral vein thrombosis, splanchnic vein thrombosis (adrenal, mesenteric); *arterial:* limb artery thrombosis, stroke, myocardial infarction, mesenteric artery thrombosis, miscellaneous artery; *microvascular*) • Necrotising skin lesions at heparin injection sites • Acute anaphylactoid reactions • Disseminated intravascular coagulation (DIC) • **Timing**: above event(s) bear(s) temporal relation to a preceding immunising heparin exposure • Absence of another more compelling explanation	**Heparin-dependent, platelet-activating IgG** • Positive platelet activation assay (e.g. SRA, HIPA) • Positive anti-PF4/polyanion-IgG ELISA (implies possible presence of platelet-activating IgG)

DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; HIPA, heparin-induced platelet activation (test); Ig, immunoglobulin; PF4, platelet factor 4; SRA, serotonin-release assay.



LIA or CLIA-IgG result (U/mL)	<1.00	1.00–4.99	5.00–15.99	≥16.00
Probability of HIT (SRA+ status) per LIA result	<1%	~30%	~70%	~90%
Probability of HIT (SRA+ status) per CLIA result	<1%	~50%	~90%	>95%

Dual LIA and CLIA analysis (6 point scale)	0	1	2	3	4	5	6
Probability of HIT (SRA+ status)	<1%	~5%	~30%	~60%	~80%	~95%	>97%
Probability of platelet-activating Abs	<3%	~10%	~55%	~85%	~98 %		

Figure 16.2 Iceberg model using published data, including two rapid assays [10].
The part of the iceberg that protrudes above the waterline indicates patients with HIT±T (HIT with or without thrombosis). The large iceberg represents the totality of antibodies detected by polyspecific PF4-dependent ELISA (ELISA-IgG/A/M), with a large subgroup comprising patients with detectable PF4-reactive antibodies of IgG class (ELISA-IgG). Almost all HIT cases are detected by SRA, with most SRA-negative HIT antibodies detectable by a modified SRA, indicated as PF4(±H)-SRA. The LIA and CLIA both have relatively high sensitivity for HIT, with the CLIA (an IgG-specific immunoassay) correlating more closely with ELISA-IgG than the LIA (which can also detect IgA and IgM antibodies). An occasional HIT case will be negative in one commercial ELISA but test positive in another. There may be occasional patients with HIT who test strongly positive by ELISA, LIA and/or CLIA, but in whom platelet-activating antibodies are difficult to detect. Importantly, the probability of HIT increases with increasing reactivity by LIA and/or CLIA. CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; HIT, heparin-induced thrombocytopenia; Ig, immunoglobulin. LIA, latex immunoturbidimetric assay; PF4, platelet factor 4; SRA, serotonin-release assay. *Source*: Warkentin 2020 [10]. Reproduced with permission of Georg Thieme Verlag KG.

- Diagnostic sensitivity for clinical HIT of the three major types of assays – ELISA-IgG/A/M, ELISA-IgG, washed platelet activation assay – is similarly high (> 99%); however, their diagnostic specificity varies: platelet activation assays > ELISA-IgG > ELISA-IgG/A/M.

Clinical Picture

Thrombocytopenia

- HIT usually results in mild to moderate thrombocytopenia (median platelet count nadir 50–60 × 10^9/L; ~90% have a nadir between 15 and 150 × 10^9/L).
- For the minority of HIT patients (~5–10%) with severe thrombocytopenia (platelet count nadir < 20 × 10^9/L), there is often associated disseminated intravascular coagulation (DIC) and risk of microvascular thrombosis.
- In > 90% of patients, the platelet count falls by > 50% from the peak platelet count that immediately precedes the HIT-associated platelet count fall.

Timing

- 'Typical-onset' HIT indicates thrombocytopenia that begins 5–10 days after an immunising heparin exposure [11].
- 'Rapid-onset' HIT refers to a platelet count fall that begins abruptly (< 24 hours) after administration of heparin or a dose increase. Almost invariably, patients have been exposed to heparin within the recent past (past 5–100 days) [11].
- HIT antibodies are remarkably transient, becoming undetectable at a median of 50–85 days (depending on the assay performed) after an episode of HIT [11]. Antibodies may become substantially weaker within a week – with platelet count recovery – even if heparin is continued [12]. This profile can be explained by marginal zone antibody-producing B cells' involvement in HIT pathogenesis (as their reactivity pattern differs from classic immunohaematological responses against alloantigens).
- 'Delayed-onset' HIT denotes thrombocytopenia that begins after the immunising heparin exposure has been stopped or that worsens after stopping heparin (onset is usually 5–10 days after the immunising heparin exposure, i.e. similar to typical-onset HIT); patient serum activates platelets *in vitro* even in the absence of pharmacological heparin (heparin-'independent' platelet activation) and has strongly positive ELISAs [7]. Such patients often have DIC. The disorder resembles a transient autoimmune reaction.
- 'Persisting' HIT refers to thrombocytopenia that is slow to recover (~1% of HIT patients take > 1 month for the platelet count to rise to > 150 × 10^9/L). In these patients, platelet numbers increase in parallel with gradually declining levels of heparin-independent platelet-activating antibodies.

Thrombosis and Other Sequelae

- HIT is strongly associated with venous and/or arterial thrombosis (relative risk 10–15).
- Thrombosis risk parallels the degree of thrombocytopenia, ranging from ~50% for patients with mild thrombocytopenia (~150 × 10^9/L) to ~90% for patients with severe thrombocytopenia (~20 × 10^9/L).
- Limb loss occurs in ~5% of patients with HIT; explanations include limb arterial thrombosis, warfarin-induced venous limb gangrene and DIC-associated microvascular thrombosis [13].
- Venous limb gangrene is acral (distal extremity) necrosis in a limb with deep vein thrombosis (DVT) that occurs despite palpable or Doppler-identifiable arterial pulses. Patients usually have a supratherapeutic international normalised ratio (INR; > 3.5) as a result of anticoagulation with a vitamin K antagonist. A prodromal state is phlegmasia cerulea dolens, i.e. an inflamed, ischaemic, painful limb [13].
- Venous predominates over arterial thrombosis (~4 : 1 ratio) [14], except in patients with arteriopathy (~1 : 1 ratio in post-cardiac/post-vascular surgery patients).

- Venous thrombotic events include (listed in descending order of frequency) venous thromboembolism (DVT > pulmonary embolism) > adrenal vein thrombosis > cerebral venous (dural sinus) thrombosis > splanchnic vein thrombosis.
- Adrenal vein thrombosis presents as unilateral or bilateral adrenal *haemorrhage*; when bilateral, death due to acute adrenal failure can occur (special relevance for critically ill patients).
- Arterial thrombotic events include limb artery thrombosis > cerebral artery thrombosis > myocardial infarction.
- Overt (decompensated) DIC occurs in 10–15% of patients with HIT, usually with platelet count nadirs < 20×10^9/L; laboratory features include relative/absolute hypofibrinogenaemia, elevated INR and/or activated partial thromboplastin time (aPTT) and (rarely) microangiopathy (red cell fragments, elevated lactate dehydrogenase, circulating normoblasts). Clinical features include microvascular thrombosis (e.g. ischaemic limb necrosis despite palpable pulses) and increased risk of treatment failure due to aPTT confounding (discussed below).
- Anaphylactoid reactions occur in ~25% of HIT patients who receive an intravenous UFH bolus and occasionally in patients administered subcutaneous LMWH (Box 16.1) [15]. There is an associated abrupt decrease in platelet count that can recover quickly after stopping heparin.

Box 16.1 Anaphylactoid reactions associated with acute (rapid-onset) heparin-induced thrombocytopenia (HIT).

Timing: onset 5–30 minutes after intravenous unfractionated heparin bolus (less commonly, following intravenous or subcutaneous low molecular weight heparin administration)

Clinical context: recent use of heparin (past 7–100 days)

Laboratory features: abrupt, sometimes rapidly reversible fall in platelet count

Signs and symptoms:

- *Inflammatory*: chills, rigors, fever and flushing
- *Cardiorespiratory*: tachycardia, hypertension, tachypnoea, dyspnoea, bronchospasm, chest pain or tightness and cardiopulmonary arrest
- *Gastrointestinal*: nausea, vomiting and large-volume diarrhoea
- *Neurological*: pounding headache, transient global amnesia, transient ischaemic attack or stroke

Pretest Probability Scores

- The '4Ts' is a pretest probability score that estimates the likelihood of HIT based upon **t**hrombocytopenia, **t**iming (of platelet count fall or thrombosis), **t**hrombosis (or other sequelae of HIT) and o**t**her causes for thrombocytopenia (Table 16.3) [16]. Low scores (3 points or fewer) are associated with < 3% probability of HIT, whereas high scores (6–8 points) indicate ~35–50% frequency of HIT.
- Another scoring system is the HIT expert probability (HEP) score [17]; like the 4Ts system, the HEP score evaluates the extent and timing of thrombocytopenia, the presence of thrombosis (or other HIT sequelae) and other potential explanations for thrombocytopenia, but assigns different numerical scores.
- Pretest probability scores are especially useful if interpreted in combination with certain immunoassays so as to predict the posttest likelihood of HIT.
- Critically ill patients with low pretest probability scores may not have HIT even if they test PF4/heparin ELISA-positive.

Laboratory Testing

Two general types of assays detect HIT antibodies: platelet activation (or functional) assays and PF4-dependent immunoassays.

Table 16.3 The 4Ts pretest probability score.

	Score = 2	Score = 1	Score = 0
***T**hrombocytopenia* Compare the highest platelet count within the sequence of declining platelet counts with the lowest count to determine the % of platelet fall **(select only 1 option)**	• > 50% platelet fall AND a nadir of > 20 AND no surgery within preceding 3 days	• > 50% platelet fall BUT surgery within preceding 3 days OR • Any combination of platelet fall and nadir that does not fit criteria for Score 2 or Score 0 (e.g. 30–50% platelet fall or nadir 10–19)	• < 30% platelet fall • Any platelet fall with nadir < 10
***T**iming (of platelet count fall or thrombosis*)* Day 0 = first day of most recent heparin exposure suspected to represent the immunizing heparin exposure **(select only 1 option)**	• Platelet fall day 5–10 after start of heparin • Platelet fall within 1 day of start of heparin AND exposure to heparin within past 5–30 days	• Consistent with platelet fall day 5–10 but not clear (e.g. missing counts) • Platelet fall within 1 day of start of heparin AND exposure to heparin in past 31–100 days • Platelet fall after day 10	• Platelet fall ≤ day 4 without exposure to heparin in past 100 days
***T**hrombosis (or other clinical sequelae)* Day 0 = first day of most recent heparin exposure suspected to represent the immunizing heparin exposure **(select only 1 option)**	• Confirmed new thrombosis (venous or arterial) • Skin necrosis at injection site • Anaphylactoid reaction to IV heparin bolus • Adrenal haemorrhage	• Recurrent venous thrombosis in a patient receiving therapeutic anticoagulants • Suspected thrombosis (awaiting confirmation with imaging) • Erythematous skin lesions at heparin injection sites	• Thrombosis not suspected

(*Continued*)

Table 16.3 (Continued)

	Score = 2	Score = 1	Score = 0
Other cause for thrombocytopenia[†] **(select only 1 option)**	• No alternative explanation for platelet fall is evident	**Possible other cause is evident:** • Sepsis without proven microbial source • Thrombocytopenia associated with initiation of ventilator • Thrombocytopenia associated with drugs	**Probable other cause present:** • Within 72 hours of surgery • Confirmed bacteraemia/fungaemia • Chemotherapy or radiation within past 20 days • DIC due to non-HIT cause • Cancer with bone marrow metastasis • Post-transfusion purpura (PTP) • Thrombotic thrombocytopenic purpura (TTP) • Platelet count < 20 AND given a drug implicated in causing D-ITP (see list) • Non-necrotising skin lesions at LMWH injection sites (presumed DTH) • Other
Drugs implicated in drug-induced immune thrombocytopenia (D-ITP) *Relatively common:* glycoprotein IIb/IIIa antagonists (abciximab, eptifibatide, tirofiban), quinine, quinidine, sulfa antibiotics, carbamazepine, vancomycin *Less common:* actinomycin, amitriptyline, amoxicillin/piperacillin/nafcillin, cephalosporins (cefazolin, ceftazidime, ceftriaxone), celecoxib, ciprofloxacin, esomeprazole, fexofenadine, fentanyl, fucidic acid, furosemide, gold salts, levofloxacin, metronidazole, naproxen, oxaliplatin, phenytoin, propranolol, propoxyphene, ranitidine, rifampin, suramin, trimethoprim			

*In some circumstances, it may be appropriate to judge timing based upon clinical sequelae, such as timing of onset of heparin-induced skin lesions.
[†]Usually, oTher scores '0 points' if thrombocytopenia is not present. However, it may be appropriate to judge oTher based upon clinical sequelae, such as whether heparin-induced skin lesions are necrotising (2 points, i.e. a non-HIT explanation is unlikely) or non-necrotising (0 points, i.e. a non-HIT explanation is likely).
DIC, disseminated intravascular coagulation; DTH, delayed-type hypersensitivity; HIT, heparin-induced thrombocytopenia; IV, intravenous; LMWH, low molecular weight heparin.
Source: Warkentin and Linkins [16]. Reproduced with permission of John Wiley & Sons.

- An unusual feature of HIT is that patient serum-/plasma-based assays are very sensitive for detecting HIT antibodies, even at the earliest phase of the platelet count decline [4].
- A characteristic feature is *inhibition* of reactivity at very high concentrations of UFH (10–100 U/mL), due to disruption of antigenic PF4/heparin complexes.
- In the absence of new clinical events (e.g. new thrombosis, new platelet count fall), a negative assay for HIT antibodies should *not* be automatically repeated a few days later; this is because a subsequent positive test result is much more likely to indicate subclinical seroconversion than 'true' HIT [4].

Platelet Activation Assays

Washed Platelet Activation Assays

- The best operating characteristics (highest sensitivity/specificity trade-off) are seen with the washed platelet activation assays, the ^{14}C-serotonin-release assay (SRA) and the heparin-induced platelet activation (HIPA) test.
- The SRA is performed in North America, using well-characterised (pedigree) donors, whereas the HIPA test is more widely used in Europe, and is usually performed with (random) donors at blood donation centres (four donors are tested separately to compensate for variable donor-dependent reactivity to HIT sera).
- Quality control manoeuvres include use of (a) negative and graded (including weak) positive HIT serum controls, (b) Fc receptor-blocking monoclonal antibodies (to confirm platelet activation occurs through platelet Fc receptors) and (c) parallel testing in a PF4-dependent ELISA (expected to be positive if the SRA or HIPA is positive).
- Sometimes, HIT antibodies fail to be detected by conventional washed platelet activation assays ('SRA-negative HIT'), but can be detected by PF4-enhanced platelet activation assays; here, external PF4 is added to the reaction well to facilitate formation of antigen on the surface of test platelets (one such assay is called the PF4-SRA) [18].
- Another PF4-enhanced functional assay uses flow cytometry to assess P-selectin as a marker for platelet activation using test platelets preincubated with PF4 [19].

Other Platelet Activation Assays

- Standard platelet aggregometry (using patient platelet-poor plasma tested against normal donor platelet-rich plasma) is not recommended, due to suboptimal sensitivity and specificity and low test/control sample throughput.
- A whole blood aggregometry assay (Multiplate®) seems to have comparable sensitivity to washed platelet assays for detecting platelet-activating HIT antibodies if a highly reactive donor is used.

Platelet Factor 4–Dependent Immunoassays (Antigen Assays)

See also Table 16.4.

Enzyme-Linked Immunosorbent Assays (Solid-Phase Assays)

- Three commercial ELISAs are available to detect anti-PF4/heparin antibodies; reference centres also offer in-house assays. ELISAs are currently the most widely used tests for HIT.
- 'Polyspecific' ELISAs detect antibodies of the three major immunoglobulin classes (IgG/A/M).
- 'IgG-specific' ELISAs are preferred because their sensitivity is as high as the polyspecific assays, with substantially greater diagnostic specificity [10].
- The magnitude of a positive ELISA result, expressed in optical density (OD) units, predicts the greater likelihood of a positive platelet activation test. For an ELISA with a positive OD range of 0.40–3.00 OD units, approximate frequencies of positive activation assays are [10]:
  - 0.40–1.00, ~5%
  - 1.00–1.50, ~20%
  - 1.50–2.00, ~50%
  - 2.00–3.00, ~90%
  - > 3.00, ~99%

Table 16.4 Platelet factor 4 (PF4)-dependent antigen assays (immunoassays).

Manufacturer	PF4 (source)	Polyanion	Assay	Ab classes
Commercial immunoassays				
ELISAs				
Diagnostica Stago (Asnières-sur-Seine, France)	Recombinant	Heparin	1. Asserachrom HIPA 2. Asserachrom HIPA-IgG	1. IgG/A/M 2. IgG
Hologic Gen-Probe (Waukesha, WI, USA)	Platelets (outdated)	Polyvinyl sulfonate (PVS)	1. PF4 Enhanced 2. PF4 IgG	1. IgG/A/M 2. IgG
HYPHEN BioMed (Neuville-sur-Oise, France)	Platelet lysate	Heparin bound to protamine	Zymutest HIA	IgG/A/M, IgG, IgA, IgM
Particle-based assays				
Milenia-Biotec (Giessen, Germany)	Platelets	Heparin	QuickLine HIT Test (lateral-flow assay*)	IgG
DiaMed (Cressier, Switzerland)	Platelets	Heparin	PaGIA	IgG/A/M
Instrumentation-based assay				
Instrumentation Laboratory (Bedford, MA, USA)	Platelets	PVS	1. HemosIL HIT-$Ab_{(PF4\text{-}H)}$ (LIA) 2. HemosIL AcuStar HIT-$IgG_{(PF4\text{-}H)}$ (CLIA)	1. IgG/A/M 2. IgG
'In-house' immunoassays (laboratories of the authors)				
Greifswald Laboratory	Platelets	Heparin	PF4/heparin ELISA	IgG, IgA, IgM
McMaster Platelet Immunology Laboratory	Platelets (outdated)	Heparin	1. PF4/heparin ELISA 2. Fluid-phase ELISA	1. IgG, IgA, IgM 2. IgG

*Also has features of a fluid-phase ELISA.
Ab, antibody; CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; HIA, heparin-induced antibodies; HIPA, heparin-induced platelet activation; HIT, heparin-induced thrombocytopenia; Ig, immunoglobulin; LIA, latex immunoturbidimetric assay, PaGIA, particle gel immunoassay.

- Diagnostic specificity is enhanced somewhat using a high heparin confirmatory step, especially at weak-positive OD values (0.40–1.00). However, at higher OD values, lack of high heparin inhibition does not necessarily rule out platelet-activating HIT antibodies.

Fluid-Phase Immunoassays

Two fluid-phase immunoassays have been described; these avoid denaturation of PF4-dependent antigens (as can occur in solid-phase ELISAs), potentially increasing diagnostic specificity.

- *Sepharose® G fluid-phase (IgG-specific) ELISA (in-house assay)*: after binding of antibodies to (5% biotinylated) PF4/heparin complexes in the fluid phase, IgG antibodies are captured using Sepharose G. After washing and incubation with streptavidin-conjugated peroxidase, the amount of immobilised biotin-PF4/heparin antibody complexes is measured using peroxidase substrate.
- *Gold nanoparticle-based fluid-phase ELISA (rapid assay)*: in this 'lateral-flow immunoassay', capillary action causes the test sample to interact sequentially with antigen (ligand-labelled PF4/polyanion complexes), then with (red-coloured) gold nanoparticles coated with anti-ligand and then with immobilised goat antihuman IgG. A positive reaction is a bold-coloured line, which can be read visually or quantitatively with an automated reader. The turnaround time of 15 minutes and the single-assay design facilitate on-demand testing.

Particle-Based Solid-Phase Immunoassays (Rapid Assays)

Particle gel immunoassay (PaGIA): this assay utilises a gel centrifugation technology where PF4/heparin complexes are bound to red, high-density polystyrene beads. Anti-PF4/heparin antibodies in patient serum/plasma bind to the antigen-coated beads; a secondary antihuman immunoglobulin antibody is added into the sephacryl gel. Upon centrifugation, agglutinated beads (with anti-PF4/heparin antibodies) do not migrate through the gel, whereas non-agglutinated beads (without anti-PF4/heparin antibodies) pass through the gel, forming a red band at the bottom. Sensitivity is lower than with the ELISAs (~95–98% versus ~99%). The diagnostic specificity is intermediate between that of the (washed) platelet activation assay and ELISA. A positive reaction at 1/4 dilution of patient serum/plasma is more specific for HIT and a positive reaction at 1/32 dilution or greater predicts the presence of platelet-activating antibodies.

Instrumentation-Based Immunoassays (Rapid Assays)

Two automated assays that utilise proprietary instruments have recently been developed:

- *HemosIL HIT-Ab$_{(PF4\text{-}H)}$*: using an analyser of the ACL TOP® family, this is a latex particle-enhanced immunoturbidimetric assay (latex immunoturbidimetric assay, or LIA) that detects anti-PF4/heparin antibodies of all classes. In this competitive agglutination assay, anti-PF4/heparin antibodies will *inhibit* the binding of an HIT-mimicking monoclonal antibody (bound to latex particles) against PF4/PVS in solution. The degree of agglutination is inversely proportional to the level of anti-PF4/heparin antibodies (assessed by a decrease in light transmittance). A positive test is a result ≥ 1.0 U/mL. The technology allows for rapid, on-demand single-patient testing.
- *HemosIL AcuStar HIT-IgG$_{(PF4\text{-}H)}$*: using an ACL AcuStar® system instrument, this is a chemiluminescence immunoassay (CLIA) that is also based upon binding of anti-PF4/heparin antibodies within patient serum/plasma to PF4/PVS. Magnetic particles coated with PF4/PVS capture anti-PF4/heparin antibodies present within a patient sample. After incubation, magnetic separation and a wash step, isoluminol-labelled antihuman IgG antibody is added, binding captured anti-PF4/heparin antibodies to the particles. After magnetic separation and a wash step, reagents that trigger the luminescence reaction are added and the emitted light is measured as relative light units (RLUs). The RLUs are

directly proportional to anti-PF4/heparin antibody concentrations and higher assay results indicate a greater likelihood of HIT.
- Both assays have higher specificity for clinically relevant anti-PF4/heparin antibodies than other antigen tests. However, the sensitivity of both assays is lower than for ELISAs (~99%): LIA ~95% and CLIA ~90–98%. By performing both of these rapid assays, the (combined) sensitivity for detecting clinically relevant HIT antibodies is ~99% [20].

Treatment

The treatment principles for strongly suspected or confirmed HIT are:

- Substitute heparin with a rapidly acting non-heparin anticoagulant, usually in therapeutic doses.
- Avoid/postpone warfarin pending platelet count recovery.
- Minimise prophylactic platelet transfusions.
- Test for HIT antibodies.
- Investigate for lower-limb DVT (e.g. with ultrasound), even if not clinically apparent.

Rapidly Acting, Non-heparin Anticoagulants

- Anticoagulants for treating HIT can be divided into (a) long-acting, indirect (antithrombin-dependent) factor Xa inhibitors (danaparoid, fondaparinux); (b) long-activity, direct (antithrombin-independent) factor Xa inhibitors (rivaroxaban, apixaban); and (c) short-acting direct thrombin inhibitors (DTIs). There is minimal experience using the oral DTI dabigatran.
- Table 16.5 compares and contrasts the indirect factor Xa inhibitors versus the DTIs for the management of HIT and suspected HIT.
- The 2018 American Society of Hematology (ASH) HIT treatment guidelines do not distinguish among the various non-heparin anticoagulants – apixaban, argatroban, bivalirudin, danaparoid, fondaparinux, rivaroxaban (listed alphabetically) – as treatment options for managing acute HIT (see Further Reading).
- HIT-associated consumptive coagulopathy can lead to treatment failure caused by systematic underdosing of the anticoagulant due to PTT confounding.

Prevention of Warfarin-Induced Venous Limb Gangrene

- Warfarin and other vitamin K antagonists are *contraindicated* during the acute thrombocytopenic phase of HIT [13]. This is because their use is strongly associated with the risk of precipitating venous limb gangrene and (less often) central necrosis of skin and subcutaneous tissues ('classic' warfarin-induced skin necrosis).
- Vitamin K should be given (5–10 mg by slow intravenous injection) if HIT is diagnosed in a patient who is receiving warfarin, especially if DTI therapy is planned (warfarin raises the aPTT and thus risks aPTT confounding of DTI therapy).
- Prothrombin complex concentrates (PCCs) contain small amounts of heparin, and thus their use is relatively contraindicated during acute HIT.
- Argatroban–warfarin overlap is problematic because argatroban prolongs the INR. In a patient who bleeds while receiving argatroban, or rivaroxaban, plasma or PCCs should not be given to reverse a very high INR because the coagulopathy is caused by argatroban rather than being because of factor deficiency.

Management of Isolated Heparin-Induced Thrombocytopenia

- 'Isolated HIT' is defined as HIT recognised because of thrombocytopenia, rather than because of a thrombotic event that draws attention to the possibility of HIT.
- Isolated HIT managed by simple discontinuation of heparin is associated with a ~50% risk of symptomatic thrombosis

Table 16.5 A comparison of two classes of anticoagulant used to treat heparin-induced thrombocytopenia (HIT).

	Indirect (AT-dependent) factor Xa inhibitors: danaparoid, fondaparinux	Direct factor Xa inhibitors: rivaroxaban, apixaban, edoxaban	Direct thrombin inhibitors (DTIs): argatroban, bivalirudin
Half-life	✓ Long (danaparoid 25 h,* fondaparinux 17 h): reduces risk of rebound hypercoagulability	✓ Long (~12 h): reduces risk of rebound hypercoagulability	Short (< 2 h): potential for rebound hypercoagulability
Dosing	✓ Both prophylactic- and therapeutic-dose regimens[‡]	✓Therapeutic-dose regimens used in case series of HIT (e.g. rivaroxaban 15 mg b.i.d.; apixaban 5 mg b.i.d.)	Prophylactic-dose regimens are not established
Monitoring	✓ Direct (anti-factor Xa levels); accurate drug levels obtained	✓ Direct (anti-factor Xa levels); accurate drug levels obtained	Indirect (aPTT): risk for DTI underdosing due to aPTT elevation caused by non-DTI factors ('aPTT confounding')
Effect on INR	✓ No significant effect; simplifies overlap with warfarin	✓ Increases INR (minor effect usually without clinical consequence)	Increases INR: argatroban > bivalirudin; complicates warfarin overlap
Reversibility of action	✓ Irreversible inhibition: AT forms covalent bond with factor Xa	Reversible inhibition	Reversible inhibition
Efficacy and safety established for non-HIT indications	✓ Treatment and prophylaxis of VTE (danaparoid, fondaparinux) and ACS (fondaparinux)[†]	✓ Treatment and prophylaxis of VTE[†]	Not established for most non-HIT settings
Platelet activation	✓ Danaparoid inhibits platelet activation by HIT antibodies (fondaparinux has no effect)	No effect	No effect
Major bleeding risk	✓ Relatively low	✓ Relatively low	Relatively high (~1% per treatment day)
Availability of antidote	No	✓Andexanet alpha	No
Inhibition of clot-bound thrombin	No effect	No effect	✓ Inhibits clot-bound thrombin
Regulatory approval to treat HIT	Danaparoid: yes (although not in the USA); fondaparinux: no	No	Argatroban: yes. Bivalirudin: no.
Drug clearance	Predominantly renal	Renal and hepatic	Variable (predominantly hepatobiliary: argatroban; predominantly renal and enzymic: bivalirudin)

Check mark (✓) indicates favourable feature in comparison of drug classes.
*For danaparoid, half-lives of its anti-IIa (antithrombin) and its thrombin generation inhibition activities (2–4 h and 3–7 h, respectively) are shorter than for its anti-factor Xa activity (~25 h).
[†]Although therapeutic dosing is recommended for HIT, availability of prophylactic-dose regimens increases flexibility when managing potential non-HIT situations.
ACS, acute coronary syndrome; aPTT, (activated) partial thromboplastin time; AT, antithrombin; b.i.d., twice a day; DTI, direct thrombin inhibitor; INR, international normalised ratio; VTE, venous thromboembolism.

(most often venous thromboembolism, VTE) and 5% risk of sudden death due to pulmonary embolism; thus, a rapidly acting alternative anticoagulant is recommended when isolated HIT is strongly suspected or confirmed.
- Our practice is to continue therapeutic-dose alternative anticoagulation until there is recovery of the platelet count to a stable plateau within the normal range; we then repeat the venous ultrasound and if it is still negative for DVT, we discontinue anticoagulation.

Adjunctive Therapies

- *Thromboembolectomy* sometimes can salvage an ischaemic limb due to acute large vessel artery occlusion by platelet-rich 'white clots'. Non-heparin anticoagulant protocols, however, are not well established for vascular surgery.
- *High-dose intravenous immunoglobulin (IvIg)* interferes with HIT antibody-induced platelet activation *in vitro* and reports indicate that its use can result in a rapid platelet count increase in HIT. The authors advocate for its use as a treatment adjunct (along with anticoagulation) in special circumstances such as severe, persisting (refractory) HIT [8] and 'spontaneous' HIT syndrome; HIT in pregnancy; and HIT complicated by cerebral vein sinus thrombosis.
- *Thrombolytic therapy* may be considered in selected patients with limb- or organ-threatening thrombosis. Concomitant anticoagulation with a non-heparin anticoagulant should be administered if heparin is part of the standard thrombolysis protocol.
- *Inferior vena cava filters* should be *avoided* because their use contributes to local thrombus formation/extension and risks underutilisation of anticoagulation, increasing risk of limb necrosis.
- Any intravascular device should be avoided, if possible, as device surfaces can trigger new thrombotic complications, and the best anticoagulants for inhibiting surface-associated thrombosis (heparin, warfarin) are contraindicated in acute HIT.

Repeat Heparin Exposure

- The immunology of HIT differs from the 'classic' immune response.
- Antibody titres decrease rapidly with cessation of HIT and in > 60% of patients, antibodies are no longer detectable after 100 days.
- In a patient with previous HIT who has become antibody negative, re-exposure to heparin only rarely results in an anamnestic immune response. If repeat immunisation occurs, at least 4–5 days are needed before antibodies are present in sufficient amounts to induce platelet activation.
- The low risk of triggering recurrent HIT allows for deliberate re-exposure to heparin for intraoperative anticoagulation during cardiac or vascular surgery. Usually, heparin is avoided before and after surgery (if antibodies are regenerated, HIT is unlikely to be retriggered in the absence of further postoperative heparin use).
- In patients who require heparin re-exposure and in whom platelet-activating HIT antibodies remain detectable, treatment strategies include (a) preoperative plasma exchange with plasma (not albumin!) replacement (to reduce HIT antibody levels usually two plasma exchange sessions are required); and/or (b) intraoperative heparin administration combined with preoperative high-dose IvIg and/or short-acting intravenous platelet inhibitor (e.g. cangrelor) [21]. To achieve sufficient platelet inhibition 2 g IvIg/kg is required, usually given at 1g/kg on two consecutive days.

Vaccine-Induced Immune Thrombotic Thrombocytopenia

- A HIT-mimicking disorder, vaccine-induced immune thrombotic thrombocytopenia (VITT; also known as vaccine-induced immune thrombocytopenia with thrombosis, and thrombotic thrombocytopenia syndrome, TTS), was

identified in early 2021 [22]; it is strongly associated with recent vaccination with an adenoviral vector vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the coronavirus that causes coronavirus disease 2019 (COVID-19).

- VITT is rare; the frequency is approximately 1–2 per 100 000 recipients of the ChAdOx1 nCoV-1 vaccine (AstraZeneca), and even less common with the Ad26. COV2.S vaccine (Johnson & Johnson/ Janssen).
- VITT pathogenesis involves formation of high-titre platelet-activating anti-PF4 antibodies that also activate neutrophils and monocytes (pancellular activation); since there is no proximate heparin exposure, VITT can be considered a subtype of spontaneous HIT syndrome (discussed above).
- VITT features usually moderate to severe thrombocytopenia (often with overt DIC), atypical and often multiple thrombotic events (e.g. cerebral venous sinus thrombosis, splanchnic vein thrombosis, adrenal haemorrhagic infarction, arterial thrombosis and VTE); onset of thrombocytopenia presumably begins approximately 5–10 days post vaccination, with symptoms of thrombosis manifesting between 5 and 45 days post vaccination (median onset of symptoms ~14 days post vaccination).
- Patient serum contains anti-PF4 antibodies readily detectable by PF4-dependent ELISAs; however, rapid HIT assays (e.g. PaGIA, CLIA, LIA) usually yield negative results. Platelet activation assays are generally positive, although PF4 supplementation may be required for optimal detection of VITT antibodies [22].
- Treatment involves anticoagulation (non-heparin anticoagulants currently favoured) and upfront high-dose IvIg (to interrupt VITT antibody-induced platelet activation); patients refractory to high-dose IvIg may require plasma exchange.

KEY POINTS

1) Heparin-induced thrombocytopenia (HIT) is a highly prothrombotic, antibody-mediated adverse effect of heparin.
2) Venous thrombosis occurs most often, especially deep vein thrombosis (DVT) and pulmonary embolism; unusual venous thrombotic events include adrenal haemorrhagic necrosis (secondary to adrenal vein thrombosis) and cerebral venous (dural sinus) thrombosis. Arterial thrombosis most often involves large limb arteries, cerebral arteries and coronary arteries.
3) The frequency of HIT varies widely and occurs more often in patients who receive unfractionated heparin (UFH) versus low molecular weight heparin (LMWH) and are postoperative (versus medical, obstetric or paediatric); there is a minor female predominance.
4) HIT is caused by immunoglobulin (Ig)G class antibodies that strongly activate platelets, triggering a procoagulant platelet response; almost always, the antibodies recognise multimolecular platelet factor 4 (PF4)/heparin complexes (the antibodies recognise one or more epitopes on PF4; heparin can be substituted by certain other polyanions).
5) Washed platelet activation assays have the highest sensitivity/specificity trade-off for detecting HIT antibodies; although PF4-dependent enzyme-linked immunosorbent assays (ELISAs) have high sensitivity for detecting HIT antibodies, they lack diagnostic specificity (except when strong positive ELISA results are observed, e.g. > 2.00 optical density units in an IgG-specific ELISA). Automated assays (latex immunoturbidimetric assay, LIA; chemiluminescence immunoassay, CLIA) have higher specificity (vs ELISAs) but slightly lower sensitivity.
6) HIT lacks features of a 'classic' immune response, i.e. antibodies of IgG class are detectable 4–5 days following an immunising heparin exposure, without preceding IgM.

7) HIT antibodies are remarkably transient, which explains why rapid-onset HIT only occurs in patients who have been exposed to heparin within the recent past. Also, it explains why heparin re-exposure is appropriate for patients with a previous history of HIT who require cardiac or vascular surgery, provided that platelet-activating antibodies are no longer detectable.
8) Vitamin K antagonists (e.g. warfarin) are contraindicated during the acute phase of HIT because their use can precipitate limb necrosis due to microthrombosis; vitamin K should be administered to a patient diagnosed with acute HIT who is receiving warfarin therapy.
9) Prophylactic platelet transfusions should be avoided during acute HIT, as thrombocytopenic bleeding (e.g. mucocutaneous haemorrhage) is not a feature of HIT and platelet transfusions in theory could increase thrombotic risk.
10) Treatment of HIT should focus on rapidly acting, non-heparin anticoagulants. There are three main classes of therapies: (a) long-acting indirect (antithrombin-dependent) factor Xa inhibitors (danaparoid, fondaparinux); (b) long-acting direct (antithrombin-independent) oral anticoagulants (apixaban, rivaroxaban); and (c) direct thrombin inhibitors (argatroban, bivalirudin).

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17 Transfusion-Transmitted Infections

Steven J. Drews[1] *and Roger Y. Dodd*[2]

[1] *Microbiology, Canadian Blood Services, Edmonton; Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada*
[2] *American Red Cross, Jerome H. Holland Laboratory for the Biomedical Sciences, Rockville, MD, USA*

Although the first written evidence of blood transfusion, albeit in dogs, is thought to have occurred in 1666 at Merton College, Oxford, it took almost 300 years for transmission of infectious agents by blood transfusion to be identified as transfusion-transmitted syphilis in the 1940s [1,2]. Around the same time point in the twentieth century, conflicts in Europe led to the emergency use of mobile blood operations, as developed by the Canadian surgeon Norman Bethune in 1930s Spain [3]. Further global conflict in the 1930s and 1940s resulted in what could now be considered public health crises; it has been suggested that the hepatitis C virus may have been widely spread during the Second World War by either unscreened blood products or non-sterile surgical procedures [4,5]. Later, in the 1960s, viral hepatitis was identified within more than 10% of blood recipients [6]. With the development of donor testing, advances in medical microbiology, infectious diseases expertise and a greater understanding of transfusion transmission risk, the estimates of residual risk at Canadian Blood Services for 2019 were 1 in 12.9 million donations for human immunodeficiency virus (HIV), 1 in 27.1 million donations for hepatitis C virus (HCV) and 1 in 1.4 million donations for hepatitis B virus (HBV) [7]. Risks will vary in different settings and over time [8–10]. Other infections have also been found to be transmitted via blood.

This chapter describes posttransfusion infection and its recognition, details the means that are used to prevent or minimise the risk of such transmission and outlines those infectious agents known to be transmitted by this route. Emerging infections are discussed in Chapter 19, and the problem of bacterial contamination of blood components is reviewed in Chapter 18.

Transmission of Infections by Blood Transfusion

Several conditions must be met for a disease to be transmitted by blood transfusion [9]:

- An asymptomatic phase during which the agent is present in the bloodstream in the donor.
- Ability of the agent to survive during the collection, processing (e.g. leucocyte reduction, fractionation) and storage (e.g. freezing) of the donation.
- Infectivity in the recipient via the intravenous route.
- A susceptible patient population (e.g. lack of immunity or frank immunocompromise).

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- Development of the disease in at least some infected recipients.

The infections discussed in this chapter are all well recognised as offering risk to transfusion recipients and subject to some measures to reduce such risk, but it must be acknowledged that, to date, no intervention has been completely effective. Residual risk, a combination of virus epidemiology and the screening test window period, still exists. In cases where testing has been implemented, any residual risk is attributable to collection of blood during the so-called early window period after exposure, when the infectious agent may circulate but be undetectable by current methods. Testing by nucleic acid testing (NAT) and/or serology has reduced this window period to a few days, lowering residual risk by many orders of magnitude [11,12]. Another threat is the development of new strains or mutations that lead to agents that escape detection from either NAT or serology, but in most cases key agents are subject to multiple redundant tests, generally avoiding this problem. This also reduces the risk attributable to laboratory failures (which are themselves very rare). In cases where the principal intervention is a donor question, it is self-evident that a donor's failure to answer the questions correctly may lead to the collection of an infectious unit. It is also not generally possible to craft a question that is completely effective in segregating all those who are infected with a given organism while assuring that there is not an undue loss of donors.

Transfusion-Transmitted Infections: Detection and Management

Clinicians responsible for the care of transfused patients should be alert to the possibility of transfusion-transmitted disease or infection. Unfortunately, recognition of most transfusion-transmitted infections (TTIs) is complicated by the following factors [9]:

- Many TTIs are asymptomatic.
- If disease symptoms occur, they tend to be non-specific (fever, flu-like illness).
- The incubation period may be prolonged, in some cases extending out to months or even years.
- The patient's underlying disease may mask or modulate evidence of other infections.
- There may be pre-existing risk factors for, or infection with, the disease agent that is thought to have been transfusion transmitted.
- Exotic infections may be transmitted by transfusion; they may be unexpected, unfamiliar, or hard to recognise or diagnose.

Effective investigation of a potential TTI is relatively complex and time-consuming and does not always lead to a definitive conclusion. Nevertheless, care should be taken to avoid inappropriate designation of the source of an infection temporally linked to transfusion.

Proper investigation of a suspected TTI includes the following:

- Clinical diagnosis of the transfusion-associated disease.
- Use of serology and/or NAT to definitively diagnose the disease and to identify the infecting agent.
- Investigation of the patient's pretransfusion blood samples to establish the absence of infection prior to transfusion.
- Investigation of the patient's risk history to eliminate the possibility of alternate routes of infection.
- Investigation of all implicated blood donors for evidence of current or recent infection with the relevant agent, with the cooperation of the blood provider.
- Comparison of the agent from patient and donor isolates by nucleic acid sequencing.
- Alerting of or consultation with infectious disease specialists and/or public health agencies as appropriate.
- Early reporting of cases to the blood provider – this is critical and is usually mandatory, so that other blood components from the implicated donor can be identified and recovered.

- Laboratory-based molecular epidemiology tools to support standard epidemiology investigations such as lookbacks.

This chapter is concerned with those infections known to be transmitted by transfusion, the individual agents responsible and the diseases that they cause. However, the presentation of a disease in a transfused patient may offer some clues. A patient may react even during administration of a blood component that is contaminated with significant levels of bacteria and/or bacterial products; this topic is discussed in Chapter 18.

Interventions to Minimise the Impact of Transfusion-Transmitted Infection

A variety of methods and processes are used to control TTIs. In general, they involve the identification of appropriate donor populations and the selection of safe donors; testing blood donations for markers of infection or infectivity; treatment of the donation; and, in some circumstances, treatment of the blood recipient. Many of these interventions are required by laws and regulations and/or by voluntary standards [13,14].

Donor populations are selected implicitly by location of collection sites and by voluntary non-remuneration policies, and explicitly by avoidance of collection from a variety of institutions (particularly prisons). Asking presenting donors questions relating to medical, travel and behavioural histories is used to assess donor suitability. These questions are intended to identify those at higher risk of certain infections. Typically, donors are asked about the following:

- A history of selected diseases or infections, such as viral hepatitis, HIV/acquired immune deficiency syndrome (AIDS), and selected parasitic diseases.
- Intimate or family exposure to specific infectious diseases.
- Exposure to blood or body fluids through illicit injection or routine transfusion.
- Receipt of potentially infectious vaccines or therapeutic agents.
- Behavioural risk factors, particularly involving male–male sex or payment or exchange of drugs for sex.
- Travel to locations or areas offering risk of exposure to (for example) malaria or the variant Creutzfeldt–Jakob disease (vCJD) prion [15].
- Use of HIV pre-exposure prophylaxis [16].

Depending upon the responses to these questions, the presenting donor will be temporarily or permanently deferred from donation and the deferral will be recorded, so that the risk may be identified should the donor try to present again during the time of deferral. The efficacy of these measures to select safer donors can be evaluated by comparing the prevalence and incidence of positive TTI test results among donors with those seen in the general population. In high-income countries, the median proportion of donations with positive or reactive results is extremely low: HIV (0.003%), HBV (0.03%), HCV (0.02%) and syphilis (0.05%) [17]. In comparison, in 2018 in the USA 1.2 million people out of a population of 327.2 million (0.37%) were living with HIV. Thus, donors have rates of infection that are several logs less than the general population.

Testing each donation for markers of infection or infectivity using serology and/or NAT is a critical step in assuring safety from infections where such tests are available and suitable; this aspect is covered in Chapter 23.

To some extent, routine post-collection processing of blood components may impact their infectivity. In general, plasma fractionation will inactivate membrane-bound viruses, bacteria and parasites. There is some evidence that infectivity for some agents may vary by component, with infectivity for malaria and *Babesia* being found primarily (but not exclusively) in red cell concentrates [18]. Conversely, after leuco reduction, infectivity for *Trypanosoma cruzi* seems to be confined to platelet concentrates [19,20], with one recent exception via red blood cells [21]. The infectivity titre for some agents

(most notably human T-cell lymphotropic virus, HTLV-1) clearly declines with product storage, although this is not considered to be a safety measure. However, leucocyte reduction of blood components clearly reduces the risk of transmission of cytomegalovirus (CMV) [22,23] and likely that of other cell-associated viruses, including HTLV [24]. Most promising, of course, is the application of formal pathogen reduction methods, which are currently available in many countries for the treatment of platelet concentrates and plasma for transfusion [25]. Methods for whole blood and for red cell concentrates remain under development.

Transfusion-Transmitted Infectious Agents

Viruses

In 2019, the International Committee on the Taxonomy of Viruses (ICTV) provided significant changes to viral taxonomy. These can be referred to on the ICTV website [26].

Hepatitis A Virus

Hepatitis A virus (*Hepatovirus A*; HAV) is a small (27–32 nm diameter), non-enveloped virus with a single strand of positive-sense RNA, 7.5 kb in length, order *Picornavirales*, family *Picornaviridae*, genus *Hepatovirus*. The primary transmission route is faecal–oral, sometimes through food or water or close personal contact. Single-source outbreaks are not uncommon. Due to outbreaks in populations that use recreational drugs or are homeless, the incidence of infection in the general population rose from less than 0.5 reported cases per 100 000 in 2011 to between 3.5 and 4.0 reported cases per 100 000 in 2018 [27]. Seroprevalence rates vary, but are estimated to be approximately 29–34% in the USA [28,29]. The incubation period is 10–50 days, with a mode of one month.

The disease presents acutely with anorexia, relatively mild fever, fatigue and vomiting, leading to typical hepatitis with varying degrees of transaminase elevation and icterus. Overall, disease tends not to be severe, with fulminant or fatal cases infrequent –usually much less than 1%. There is a 7–14-day period of viraemia prior to the appearance of symptoms and during this time blood is likely to be infectious via transfusion. Tests for immunoglobulin (Ig)G and IgM antibodies and for viral RNA are available.

A handful of cases of transfusion-transmitted HAV cases have been reported, some with secondary transmission [30]. Testing of whole blood donations is not warranted because transmission is so rare, but plasma for further manufacture is tested for HAV RNA by pooled NAT. Blood donors are usually asked to notify the collection site if they become sick shortly after donation, and such post-donation information has led to the identification and recovery of at least some potentially infectious units.

Hepatitis B Virus

Hepatitis B virus (HBV) is an enveloped spherical virus 42–47 nm in diameter, with a partially double-stranded, circular DNA genome 3.2 kb in length with overlapping reading frames, Order *Blubervirales*, family *Hepadnaviridae*, genus *Orthohepadnavirus*. Transmission routes are primarily sexual, parenteral and perinatal. An unusual feature of HBV infection is the overproduction of excess viral coat material that can circulate at high concentrations, termed hepatitis B surface antigen (HBsAg); its presence is indicative of active infection (acute or chronic) and it is the primary analyte for blood donor screening. Antibodies to HBsAg (anti-HBs) are generally indicative of past infection, but antibodies to the inner core of the virus (anti-HBc) appear earlier and may also indicate some risk of infectivity. IgM anti-HBc in combination with HBsAg are markers of infection within the last six months [31]. Detectable HBV DNA in the plasma is associated with varying levels of infectivity, depending upon the phase of

the infection; the early window phase when DNA is the only detectable marker appears to be the most infectious.

In 2018, the estimated incidence of acute infection in the USA was approximately 1 per 100 000, whereas the prevalence of any HBV infection was 4–5% [27,32]. A decrease in the prevalence of past or present infection from 1999–2002 (5.7%) to 2015–2018 (4.3%) occurred while evidence of vaccination increased in adults from 12.3% (1999–2002) to 25.2% (2015–2018). In 2015, the World Health Organization estimated that 257 million people were living with chronic HBV infection globally [33].

The incubation period from exposure to infection is from one week to six months. While many infections are asymptomatic, the range of disease manifestations is extensive, from mild acute symptoms to life-threatening or fatal fulminant cases. Symptoms are generally like those described for HAV infection. Chronic infection results more frequently from infection early in life and chronic disease may lead to cirrhosis and/or liver cancer. Diagnostic tests include serum transaminase measurement and detection of HBV antibodies, particularly IgM anti-HBc. NAT may also be of value.

A review of blood screening benefits is included in a review by Candotti and Laperche in Further Reading.

Hepatitis C Virus

Hepatitis C virus (*Hepacivirus* C, HCV) is a small, enveloped spherical (55–65 nm diameter) virus with a single positive strand of RNA, 9.6 kb in length, order *Amarillovirales*, family *Flaviviridae*, genus *Hepacivirus*. The transmission route is primarily parenteral. Between 2013 and 2017, the incidence of new reported infections in the USA ranged from 0.7 to 1.0 per 100 000 annually [34]. The incidence has declined significantly over recent years. Most infections are chronic and lifelong; around 20% of infections may resolve. The incubation period is typically 4–12 weeks, with an extended range of 2–24 weeks. Most infections are asymptomatic, but when symptoms occur, they include fever, fatigue, loss of appetite and abdominal pain. Diagnostic tools include serum transaminase testing, antibody and RNA detection. After many years, chronic disease may lead to cirrhosis and, in some cases, liver cancer.

Although the virus was not specifically identified until 1989, it was recognised as the predominant causative agent of post-transfusion hepatitis [6]. Interventions to reduce the transmission of HCV by transfusion include questioning donors about a history of viral hepatitis or exposure to a case, and about risk behaviours involving parenteral exposure to blood. All donations are tested for antibodies to HCV and in some cases also to the core antigen of the virus. The development, progressive improvement and universal implementation of tests for anti-HCV in donors have profoundly reduced transmission, with a further significant improvement attributable to the implementation of testing for HCV RNA [35]. NAT and serology, in the setting of low incidence, are now associated with short window period risk-day equivalents (2.9 days, 95% confidence interval 3.6–4.6) and very low residual risks for transfusion transmission (1 in 12.6 million).

Hepatitis D Virus

Hepatitis D virus (*Hepatitis delta virus*, HDV) is a very small RNA virus in the genus *Deltavirus* that only infects those with ongoing HBV infection. HDV co-infection increases the severity of disease in those with chronic hepatitis B [36]. Because HDV is dependent on HBV for replication, measures to prevent HBV transmission are also effective against HDV.

Hepatitis E Virus

Hepatitis E virus (*Orthohepevirus A*; HEV) is a small, non-enveloped icosahedral (30–34 nm diameter) virus with a single, positive strand of RNA, 7.2 kb in length, Order *Hepelivirales*, family *Hepeviridae*, genus *Orthohepevirus*. There are four major

serotypes with varying geographical distribution and pathogenicity for humans. The transmission routes for types 1 and 2 are primarily faecal–oral, often water-borne, but types 3 and 4 are zoonoses that are frequently food borne, with cases attributable to consumption of raw or undercooked pork [36]. A recent review article by Goel et al., listed in Further Reading, describes the prevalence of HEV viraemia among blood donors. Transfusion transmission has been noted rarely in non-endemic areas, and with some frequency in endemic areas, such as Hokkaido in Japan [36]. NAT of presenting donors has been implemented as a preventive measure in some areas of concern and, in some cases, for plasma for further manufacture, but there seems to be little enthusiasm for widespread application of this intervention.

Human Immunodeficiency Viruses 1 and 2

Human immunodeficiency viruses (human immunodeficiency virus-1/2; HIV-1/2) are enveloped, predominantly spherical (106–183 nm diameter) viruses, with two linear, positive-sense strands of RNA, 9.2 kb in length, order *Ortervirales*, family *Retroviridae*, subfamily *Orthoretrovirinae*, genus *Lentivirus*. There are two major species, HIV-1 and HIV-2, although HIV-2 is much less common and less virulent than HIV-1. HIV-1 has multiple distinct clades [36]. The predominant transmission routes are sexual, perinatal and parenteral. Prevalence and incidence rates vary widely, with national rates varying from < 1% to slightly higher than 20% in sub-Saharan Africa [37]. Prevalence in the USA aged ≥ 13 years for people living with diagnosed and undiagnosed HIV is estimated at about 427.5 per 100 000 annually (2018). Incidence rates in the USA aged ≥ 13 years are estimated to be about 5.2 per 100 000 annually (2018) [38]. Rates in Western Europe and high-income countries are generally somewhat lower. The incubation period of the acute retroviral syndrome averages about 21 days, but may range from 5 to 70 days. It is now known that HIV RNA becomes detectable around 9 days after infection using current NAT assays. Sensitive tests for antibodies to HIV-1/2 as well as HIV have shortened the diagnostic window period from 3–4 weeks (antibody only) to 2 weeks [39]. Dual screening of donations with serology (without p24) assays as well as NAT has shifted the HIV window period, risk-day equivalents, down to 6 days in some settings [35].

Most infections are asymptomatic and the acute retroviral syndrome, when it occurs, tends to be relatively mild, with a short (a week or two) period of fever, fatigue and possibly lymphadenopathy and rash. Typically, patients recover and are asymptomatic for many years thereafter, until the symptoms of full-blown AIDS emerge. Diagnosis of infection may be based upon tests for antibodies to HIV and/or the presence of HIV RNA in the plasma.

In Canada, during 2012–2014, a period where all blood donations were screened with HIV-1/2 serology and NAT minipools, the residual risk for HIV was estimated at 1 per 21.4 million donations [35]. Again, however, transfusion-transmitted HIV infections are not recognised as frequently as this risk estimate would suggest. It is of interest to note that two of the five transmission events noted in the USA since 1999 have involved infection from a transfusable plasma unit, but not from the accompanying red cell concentrate, suggesting that the sensitivity of NAT is approaching the infectious dose of HIV offered by a red cell concentrate [12,40].

While the AIDS epidemic has been a medical and human disaster, it stimulated the current stringent approach to blood safety and continuous quality improvement. All donors are directly asked about a history of AIDS-related symptoms and about possible exposure to infection. Questions about behavioural risk are asked and individuals acknowledging such risk are permanently or temporarily deferred. Some of the questions, particularly those relating to male–male sexual activity, have been challenged as discriminatory.

Human T-Lymphotropic Viruses 1 and 2

Human T-lymphotropic viruses (primate T-lymphotropic virus-1/2; HTLV-1/2) are enveloped, spherical (150–200 nm diameter) viruses, with two linear, positive-sense, single strands of RNA, 8.5 kb in length, family *Retroviridae*, genus *Deltaretrovirus*. There are two different viruses, HTLV-1 and HTLV-2, and at least two additional variants have been described. The primary routes of transmission are sexual and perinatal (via breast milk), and parenteral transmission (particularly via injecting drug use) has been widely documented, especially for HTLV-2. Prevalence rates vary widely, but tend to be very low in developed Western countries. There are pockets of high prevalence in Japan, the Caribbean and Africa (HTLV-1) and among some native populations in the Americas (HTLV-2). Infection is most often asymptomatic; for HTLV-1, there is a lifetime risk of a few percent for the eventual development of adult T-cell leukaemia/lymphoma or tropical spastic paraparesis, but only the latter has ever been associated with transfusion. Less is known about disease associations for HTLV-2.

Transfusion transmission of these viruses has been recognised for many years; however, leucocyte reduction seems to eliminate the risk of transmission, which also declines during refrigerated storage of red cell components. In one Canadian study, with universal leucocyte reduction and universal testing, the residual risk for releasing a potentially infectious unit containing HTLV was modelled at 1 in 1.2 billion units [41].

Cytomegalovirus

Human cytomegalovirus (*Human betaherpesvirus 5*; CMV) is an enveloped, spherical beta herpes virus 200–300 nm in diameter, with a double-stranded DNA genome of 235 kb pairs, order *Herpesvirales*, family Herpesviridae, subfamily *Betaherpesvirinae*, genus *Cytomegalovirus*. Seroprevalence rates vary by age and location, but are of the order of 30–40% among blood donors in the USA and Western Europe [36,42]. There are no good measures of incidence rates, but the presence of antibodies to CMV implies that virus is present, albeit often in latent form. The normal transmission route is by contact, droplets or body fluid exposure, but it is also transmissible from mother to fetus and by blood transfusion, breast milk and organ transplant [43,44]. In general, healthy individuals are asymptomatic or show only mild symptoms (fever, lymphadenopathy, mononucleosis-like disease), but vulnerable individuals, including the fetus, low-birthweight infants, transplant patients and those with severe immune deficiencies, may suffer serious or fatal disease, including pneumonia, multiorgan disease, etc. Infection during pregnancy may have profound effects on the fetus, including developmental problems.

TTI is typically recognised 1–2 months after transfusion. There are two primary interventions to reduce or eliminate post-transfusion CMV infection: leucocyte reduction and/or the use of seronegative blood components for those at risk. Studies have suggested that both methods have similar efficacy in reducing risk, but that breakthrough infections may still be seen [22,45]. Explanations for such breakthrough cases include failure of testing or leucocyte reduction, the presence of extracellular virus in early infection and the possibility that at least some hospital-acquired infections may be mistaken for transfusion transmissions. Breastmilk may be the primary cause of postnatal CMV infection in very low-birthweight infants, especially when CMV-seronegative and leuco-reduced blood products are transfused [43]. However, reactivation of latent CMV infection triggered by transfusion may account for most reported cases of transfusion-transmitted CMV [46]. Finally, with leucocyte reduction, the emphasis on the provision of CMV-safe blood products is rapidly diminishing, and in some cases these units are only maintained for use in intrauterine transfusions [47].

Other Human Herpes Viruses

Two other human herpes viruses, Epstein–Barr virus (*Human gammaherpesvirus 4*; EBV)

and human herpes virus 8 (*Human gammaherpesvirus 8*; HHV-8), are known to be transmissible by transfusion [36]. EBV is an almost ubiquitous virus, associated with infectious mononucleosis and with Burkitt's lymphoma in Africa and nasopharyngeal carcinoma in Asia. Evidence is also increasing for its role in the causation of lymphoproliferative disease among transfused immunocompromised patients, but other than leucocyte reduction, there is little in the way of an intervention, in the absence of pathogen reduction. HHV-8 is the causative agent of Kaposi's sarcoma and there is evidence for its transmissibility by transfusion in Africa [48]. To date, however, there does not appear to be any evidence for transfusion-transmitted HHV-8 disease; reductions in viral loads likely are the result of leucocyte reduction [36].

West Nile Virus

West Nile virus (WNV) is an enveloped, spherical (40–60 nm diameter) virus with a linear, single strand of positive-sense RNA, 11 kb in length, family *Flaviviridae*, genus *Flavivirus* (Japanese encephalitis complex). The primary transmission route is via (mainly culicine) mosquitoes and the amplifying hosts are primarily birds. Humans are accidental, dead-end hosts, although the virus is readily transmitted via blood transfusion [49]. At the peak of the epidemic in the USA in 2002, several hundred thousand individuals were naturally infected each year. Since then the number of cases has declined and appears to have reached stability. The virus is endemic in parts of Africa, the Middle East and parts of southern Europe, where smaller outbreaks frequently occur.

A summary of the presentation of this disease by Zou et al. is included in Further Reading. In the USA, cases occur mainly between April and October. In 2002, there was a report of 23 well-characterised transfusion-transmitted cases of WNV infections and within less than a year, universal donor screening for WNV RNA by pooled NAT was implemented in the USA [50]. Such testing did not detect all infectious donations, and single-donation testing was introduced in areas and at times with a high incidence of WNV activity [51,52]. Outside the USA and Canada there has not been widespread testing, but donor deferral based upon travel to endemic areas has been implemented in some countries. Testing has been implemented in parts of Europe in response to several discrete outbreaks.

Other Arboviruses

WNV illustrated two somewhat unexpected facts: the ability of arthropod-borne viruses (arboviruses) to establish huge, unprecedented outbreaks in previously unaffected areas; and efficient transmission of an acute infection via blood transfusion. Accordingly, unexpected intense outbreaks of infection with Chikungunya virus (an alphavirus transmitted by *Aedes* spp. mosquitoes) resulted in specific measures designed to prevent transfusion transmission in some affected areas; however, although explosive outbreaks occurred, transfusion transmission was never documented [13,53].

In contrast, seven clusters of transfusion transmission of dengue virus have been reported from Hong Kong, Singapore, Brazil and Puerto Rico [54]. Donor testing has been implemented in Puerto Rico as well as French Polynesia and may be considered elsewhere in the future, particularly in countries in which this virus is not endemic but where outbreaks may occur [55].

Zika virus

Zika virus is a mosquito-borne flavivirus closely related to dengue viruses that was responsible for an outbreak in the Americas in 2015 and 2016. As of July 2019, 87 countries had current or previous Zika virus transmission, with over 50% of those countries in the Americas [56]. However, by February 2020 there were no countries with active Zika virus transmission [57]. Zika virus has been proven to cause fetal loss, a congenital Zika virus–related syndrome including microcephaly and Guillain–Barré syndrome and other neurological complications in adults.

However, in most cases (~80%) Zika virus infection is asymptomatic.

In May 2021, the US Food and Drug Administration (FDA) withdrew the guidance document titled 'Revised recommendations for reducing the risk of Zika virus transmission by blood and blood components'. The FDA's decision document is listed in Further Reading.

Human B19 Parvovirus (B19V)

B19V is a small, non-enveloped, icosahedral (23–26 nm diameter) virus with a linear, negative-sense, single strand of DNA, 5.6 kb in length, in the family *Parvoviridae*, genus *Erythrovirus*. The primary transmission routes are respiratory and transplacental. The virus is transmitted by blood transfusion and, at least in the past, via some plasma-derived products. Levels of viraemia during acute infection can be extremely high, sometimes exceeding 10^{12} DNA copies per mL. The virus is ubiquitous, often causing seasonal outbreaks of mild disease, particularly among children. Seroprevalence rates are around 50% in adults and the incidence rate can be 1.5% [13].

Most infections are asymptomatic, but the virus causes erythema infectiosum (fifth disease) in children and occasional arthropathy in adults. Of concern is transient aplastic crisis in patients with shortened red blood cell (RBC) survival or haemolytic anaemias; in some cases there may be pure red cell aplasia or pancytopenia. Infection in pregnant women may result in hydrops fetalis. Symptomatic infection from transfusion transmission is extremely rare. Nevertheless, in some countries (such as Germany and Japan) donor blood is routinely screened for viral DNA or by haemagglutination to eliminate the transfusion of components with high titres of virus. Currently, plasma for further manufacture is tested in pools for B19 DNA to minimise the levels of virus in manufacturing pools. Such testing is becoming available through rapid, high-throughput procedures and this may lead to expansion of routine blood donation testing.

SARS-CoV-2

SARS-CoV-2 is a single-stranded, positive-sense RNA virus that falls within the viral species designated as *severe acute respiratory syndrome-related coronavirus*. Taxonomically, this virus is classified within the order *Nidovirales*, suborder *Cornidovirineae*, family *Coronaviridae*, subfamily *Orthocoronavirinae*, genus *Betacorovavirus* and subgenus *Sarbecovirus* [58]. The World Health Organization provides up-to-date epidemiological reports on this virus, identified in Further Reading. Although the virus causes a respiratory illness, a variety of other sequelae have been described. SARS-CoV-2 mRNA has been detected in blood products of ill patients and some blood donors; however, a review of the data and literature reviews in 2020 could not identify a signal for transfusion transmission of SARS-CoV-2 [59–63]. As of August 2021, there have been no descriptions of transfusion transmission of SARS-CoV-2.

Bacteria

Currently, the major blood safety risk from bacteria results from contamination of components and subsequent outgrowth, resulting in septic reactions in the patient. This is discussed in Chapter 18. There has been no reported case of transfusion-transmitted syphilis in the literature since 1960. The rarity of such transmission is likely due to a combination of factors, including donor selection and testing and the fragility of *Treponema pallidum* (the infectious spirochete) in stored components, along with the frequent use of antibiotics among patients.

There have been concerns about the potential for transmission of Q fever (caused by the small bacterium *Coxiella burnetii*) because of large, focused outbreaks of human infection in The Netherlands from 2007 to 2009. In those outbreaks, infections resulted from human exposure to airborne bacteria associated with intensive goat farming. Investigations demonstrated bacteraemia in some patients and a small amount of

suggestive evidence for rare transfusion transmission. In times and areas of concern, donations were tested for *C. burnetii* by polymerase chain reaction (PCR) [64]. Veterinary public health measures have, however, essentially eliminated the outbreaks. By 2014, chronic infectious *C. burnetii* was not considered to be a risk to the blood supply [65]. Other tick-borne *Rickettsia*-like bacteria have engendered some concern, and to date there have been at least eight reports of transfusion transmission of *Anaplasma phagocytophilum* [36]. There has been no evidence of transmission of *Borrelia burgdorferi* (the agent of Lyme disease) by this route [36].

Parasitic Diseases

Malaria

Human malaria is caused by intraerythrocytic protozoan parasites of the genus *Plasmodium*, namely *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*; some cases have also been attributed to the primate parasite *P. knowlesi* [36]. The parasites are transmitted by *Anopheles* mosquitoes. The parasites may be present in the circulation during a prolonged asymptomatic period and are readily transmitted by blood transfusion. Such transmission is thought to be quite common in endemic areas in the tropics, but is also a significant risk in non-endemic countries, because of collection of blood from donors infected via travel from endemic areas. Disease symptoms include periodic fever, rigors and chills, headache, myalgias, arthralgias, splenomegaly and haemolytic anaemia. Although the normal incubation period is usually a few weeks, this period may be extended in blood recipients and recognition and diagnosis may not be easy. In general, the most severe forms of the disease are attributable to *P. falciparum*.

Diagnosis may be achieved through microscopic inspection of blood smears and serological testing; research-level NATs are also available. In non-endemic countries, transfusion-transmitted malaria is controlled by questioning donors about a history of malaria and of travel from or residence in malarious areas. Policies differ somewhat, but in general, casual travel by residents of non-endemic countries is not a major risk, provided that such travellers are deferred for a few months. On the other hand, those who have resided for long periods in malarious areas may be partially immune and can be infectious for several years. Many donors are deferred for travel histories, with a negative impact on blood availability; in some European countries and in Australia, deferred donors may be selectively tested for antibodies to *Plasmodium* spp. and, if non-reactive, are permitted to donate after a shortened deferral period [66].

Babesiosis

Babesia is also an intraerythrocytic protozoan parasite and the causative agent of babesiosis; a variety of species may be found throughout the world [18]. *Babesia* spp. are transmitted by ticks and primarily affect mammals, with humans as an accidental host. Babesiosis has symptoms like those of malaria and may be severe in the elderly and asplenic patients. An excellent review of transfusion-transmitted *Babesia* is included in Further Reading. In the USA since 1979, there have been 200 cases of transfusion-transmitted babesiosis (including 3 from *B. duncani*) [67]. Few cases have been reported from any other countries. The disease is generally treatable, but nevertheless transfusion-transmitted cases have a significant fatality rate. Donor questioning regarding tick bite or clinical disease is insensitive. In 2019, the FDA released the document 'Recommendations for reducing the risk of transfusion-transmitted babesiosis', which is cited in Further Reading.

Chagas' disease

Chagas' disease is caused by the protozoan parasite *Trypanosoma cruzi*, which infects numerous mammalian hosts and is transmitted to humans by reduviid bugs [36]. The

parasite is endemic in the Americas, generally between latitudes 40° N and 40° S. Most human infections occur in rural areas of Latin America where the vector insects colonise substandard housing. An excellent review by Bern et al. of this parasite, its diagnosis, its epidemiology and pathogenesis is included in Further Reading.

Prevention of transfusion transmission relies primarily upon blood donor testing for antibodies to *T. cruzi*. Such testing is widespread in Latin America and was implemented in the USA in 2007. Subsequent evaluation of the testing programme suggested that selective testing was effective; currently, blood donors are tested only once and if non-reactive, subsequent donations are accepted without any testing. In other countries, notably Canada and Spain, presenting donors are asked about prolonged travel, residence or birth in Chagas-endemic countries and whether their mothers or grandmothers were born in such areas. If so, the donors are tested for *T. cruzi* antibodies and may donate if such tests are non-reactive.

The number of transfusion-transmitted cases is limited and has been described primarily from platelets [68], although there has been a single report of transmission from a red cell concentrate [21].

Prions

Variant Creutzfeldt–Jakob disease

Variant CJD is the human form of bovine spongiform encephalopathy (BSE, mad cow disease), transmitted to humans through ingestion of tissues from infected cattle. The disease was first recognised as a distinct entity in 1996. Although like classic CJD, vCJD occurs primarily among younger individuals, presents with psychiatric symptoms and generally has a longer course from diagnosis to death [13]. The pathology typically involves unusual, florid plaques in the brain. About 226 cases have occurred, mostly in the UK. The frequency of reported cases has been declining over the past 10 years.

Careful review of surveillance data has revealed four instances of transmission of the vCJD agent by transfusion, all in the absence of leuco reduction and prior to the use of more stringent/complex industrial plasma fractionation approaches [69]. A review by Hewitt et al. is highlighted in Further Reading. In the USA, protective measures include permanent deferral from donation of individuals judged at risk of exposure to BSE by residence or prolonged cumulative travel to the UK and Western Europe, and similar measures have been taken elsewhere. To date, there has been no evidence that the classic form of CJD is transmissible by transfusion [70].

KEY POINTS

1) Several pathogens, including viruses, bacteria, protozoan parasites and one prion, are known to be transmitted by transfusion.
2) Measures are in place to control such transmission, including blood donor selection, deferral, laboratory testing and component treatment.
3) These measures have reduced the incidence of key transfusion-transmitted infections to very low levels, usually less than one case per million components transfused.
4) Transfusion-transmitted infections are difficult to detect and diagnose.
5) Careful studies involving the patient and all implicated donors are necessary to confirm that an infection is attributable to transfusion.
6) Transfusion-transmitted infection should be appropriately reported to blood providers and other agencies, as required by regulation or practice.

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18 Bacterial Contamination

Sandra Ramírez-Arcos[1,2] *and Mindy Goldman*[3]

[1] *Medical Affairs and Innovation, Canadian Blood Services, Ottawa, Ontario, Canada*
[2] *Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada*
[3] *Canadian Blood Services; University of Ottawa, Ottawa, Ontario, Canada*

Incidence of Bacterial Contamination

Transfusion-associated septic events have been reduced by the introduction of improved donor screening and skin disinfection methods, as well as implementation of first aliquot diversion and bacterial testing. Recent reports of septic reactions indicate that bacterial contamination of blood components continues to be the predominant transfusion-associated infectious risk in Europe and North America [1,2]. From 2015 to 2019, the US Food and Drug Administration (FDA) reported 20 fatalities caused by blood components contaminated with bacteria [2]. In Canada, the Transfusion Transmitted Injuries Surveillance System Programme Report for 2014–18 described 31 transfusion reactions involving bacterially contaminated blood components [3]. Between 2011 and 2019, the UK's Serious Hazards of Transfusion (SHOT) programme documented nine near-misses and one confirmed septic transfusion reaction involving a platelet unit contaminated with *Staphylococcus aureus* [4].

The frequency of bacterial contamination in platelet concentrates varies broadly within countries. For example, while the Australian Red Cross reported a rate of confirmed and indeterminate results of 0.22% for pooled platelets concentrates and 0.11% for apheresis units, respectively [5], Canadian Blood Services documented a frequency of 0.09% and 0.04% confirmed-positive results for platelet pools and apheresis units, respectively [6]. The US National Healthcare Safety Network (NHSN) Hemovigilance Module collected information from 195 facilities from 2010 to 2016 and reported rates of septic reactions of 2.5/100 000 (0.25%) and 0.9/100 000 (0.09%) for apheresis and whole-blood-derived platelet concentrates, respectively [7].

Blood Components Implicated in Adverse Transfusion Reactions

Platelet Concentrates

Platelet concentrates (PCs) are the blood components most susceptible to bacterial contamination due to their storage conditions being amenable for bacterial growth. PCs have a physiological pH and high glucose content and are stored with constant agitation at 22±2 °C in gas-permeable plastic containers. The initial levels of bacteria in PCs are usually exceedingly low (< 10 colony forming units, cfu/PC unit), but clinically significant levels (10^5 cfu/mL) can be reached after 3–5 days of storage, depending on the organism [8].

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Clinical sequelae of transfusing bacterially contaminated PCs are variable and may be acute or delayed, depending on the severity of the recipient's medical condition, the type and concentration of the contaminant organism and the timing of transfusion [1,5–9].

Red Blood Cells

Storage of red cell units at low temperatures (1/2–6 °C) limits bacterial growth and decreases the risk of adverse posttransfusion events. However, psychrotrophic (growing optimally at refrigeration temperatures) pathogenic bacteria can proliferate under red cell storage conditions, reaching clinically significant concentrations [10].

Reactions associated with transfusion of bacterially contaminated red cell units are usually severe, due to infused endotoxin (lipopolysaccharide) associated with the cell wall of Gram-negative bacteria. Clinical symptoms may include fever over 38.5 °C, hypotension, nausea and vomiting starting during the transfusion. Septic shock with complications such as oliguria and disseminated intravascular coagulation may occur [11].

Plasma and Cryoprecipitate

The incidence of adverse transfusion reactions (ATRs) due to contaminated plasma or cryoprecipitate is low, with only a few reports of transfusion-transmitted infections involving contaminated plasma documented in recent literature [2,7,12].

Contaminant Bacterial Species

Platelet Concentrates

Gram-positive bacteria are the predominant PC contaminants. Although these bacteria can survive and proliferate during PC storage, most of them are not considered to be pathogenic. Coagulase-negative staphylococci such as *Staphylococcus epidermidis* and propionibacteria are the predominant contaminants of PCs [2,5–8]. Transfusions with fatal outcomes due to platelets contaminated with *S. epidermidis* have been reported worldwide [2,13]. Missed detection of *S. epidermidis* is attributed to slow growth under platelet storage conditions and the ability to form slimy bacterial aggregates (biofilms) [13]. Other Gram-positive bacteria often identified as PC contaminants include corynebacteria, *S. aureus*, *Bacillus* spp. and *Streptococcus* spp. [1,2,5–9]. Most of the PC contaminants are either aerobic or facultative anaerobic bacteria, with *S. aureus* and streptococci being mostly implicated in transfusion reactions. There have also been reports of septic reactions associated with strict anaerobic organisms such as *Clostridium perfringens* [1].

Gram-negative bacteria can also be present in PCs and will cause severe and often fatal infections due to the potent septic shock reaction-induced endotoxins, which elicit an uncontrolled inflammatory response in the recipient. The most frequently identified Gram-negative PC contaminants include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp. and *Serratia* spp. [2,5–9].

Red Blood Cells

Red cells are the most frequently transfused blood component. The predominant red cell contaminants are Gram-negative bacteria of the family Enterobacteriaceae, with *Yersinia enterocolitica* being the predominant species. *Yersinia* is a psychrotrophic organism and proliferates well at 1–6 °C, reaching levels > 10^8 cfu/mL after three weeks of incubation. Transfusion of red cell units heavily contaminated with *Y. enterocolitica* results in severe septic shock due to high levels of endotoxin [10].

Other red cell Gram-negative bacterial contaminants include *Serratia* spp., *Pseudomonas* spp., *Enterobacter* spp., *Campylobacter* spp. and *E. coli*, all of which have the potential to cause endotoxic shock in recipients [2,4,10].

Plasma and Cryoprecipitate

Burkholderia cepacia (previously known as *Pseudomonas cepacia*), *Pseudomonas aeruginosa* and more recently coagulase-negative *Staphylococcus* and *Bacillus* have been implicated in ATRs due to contaminated plasma and cryoprecipitate [2,7,12].

Sources of Contamination

Contaminant bacteria of blood components can originate from the donor or the blood collection and processing procedures.

Blood Donor

The predominant blood component bacterial contaminants are Gram-positive bacteria that are part of the normal skin flora and, more rarely, Gram-negative bacteria that can originate from silent donor bacteraemia or be part of the transient skin flora. It is impossible to completely decontaminate human skin and it has been reported that normal skin flora organisms such as *S. epidermidis* can adhere firmly to human hair despite skin disinfection [2,6–8].

Asymptomatic donor bacteraemia may lead to contamination of blood components. Low-level bacteraemia may occur in the incubation or recovery phase of acute infections after procedures such as tooth extraction. Chronic, low-grade infections, such as osteomyelitis, have been associated with contaminated platelet products, as have gastrointestinal disorders such as diverticulosis and colon cancer [14].

Blood Collection and Production Processes

Blood collection and production processes can also be sources of bacterial contamination. Three cases of *Serratia marcescens* sepsis following platelet transfusions were linked to contaminated vacuum tubes used for blood collection [15]. A recent report of a fatal septic reaction due to PC storage container leakage highlights the risk of postmanufacturing environmental contamination of PCs [16].

Investigation of Transfusion Reactions

Symptoms of transfusion-associated septic reactions usually appear during the first four hours after the transfusion was initiated; however, there are reports of delayed symptoms of a transfusion reaction that could go under-recognised [13]. If a septic reaction is suspected, the transfusion should be stopped immediately. Remaining components, intravenous solutions and blood samples from the recipient should be sent for microbiological investigation. Septic transfusion reactions are confirmed if the same bacterium is isolated from the recipient and the implicated blood component. Associated components to the concerned blood component should be recalled and, if available, also cultured. If the contaminant organism is not part of the normal skin flora, the donor should be contacted and followed up. The donor's health should be considered as well as the possibility of recurrent contaminated donations. Depending on the results of the investigation, donor deferral from future donations might be required [17].

Prevention Strategies

Strategies used to decrease the levels of bacterial contamination in blood components include donor screening, skin disinfection, first aliquot diversion, pretransfusion detection and pathogen reduction technologies [17]. Recently, the FDA issued a guidance for industry outlining recommendations for blood collection services and transfusion services to enhance PC safety (Table 18.1) [18].

Table 18.1 Summary of Food and Drug Administration (FDA) guidelines to enhance platelet concentrate (PC) safety [18].

PC shelf-life	Approach	PC product	Sampling volume†	Sampling time	Post-sampling quarantine
≤ 5 days	Culture	Pre-storage whole-blood derived (WBD) pools or apheresis units*	16 mL	≥ 36 hours	≥ 12 hours
		Apheresis units*	16 mL	≥ 48 hours	≥ 12 hours
		Single WBD pools	8 mL	≥ 36 hours	≥ 12 hours
	Rapid test	Single apheresis units		As per vendor's instructions	
		Single pre-storage WBD pools			
	Pathogen reduction	Apheresis units			
Up to 7 days‡		Apheresis units*	16 mL	≥ 48 hours	≥ 12 hours
	Step 1 Culture *AND*	Pre-storage WBD pools *OR* apheresis units	16 mL	≥ 24 hours	≥ 12 hours
	Step 2 Culture		≥ 8 mL	≥ 4 days	
	Step 1 Culture *AND*	Apheresis units	16 mL	≥ 36 hours	≥ 12 hours
	Step 2 Culture		16 mL	≥ 4 days	
	Step 1 Culture *AND*	Pre-storage WBD pools *OR* apheresis units	16 mL	≥ 24 hours	≥ 12 hours
	Step 2 Rapid test			As per vendor's instructions	

*Sampling of each unit. If the apheresis unit will be split, each split unit should be tested.
†PC samples are inoculated equally into aerobic and anaerobic culture bottles (16 mL) or into one aerobic culture bottle (8 mL).
‡Bacterial testing used to extend PC shelf-life beyond day 5 for a maximum of 7 days should be performed with devices labelled with 'Large Volume Delayed Sampling (LVDS)' as an acceptable safety measure, and PC storage containers must be cleared or approved by the FDA for 7 days of storage.

Donor Screening

Most blood centres have established methods for donor screening to avoid collection of potentially contaminated blood components. Donor screening includes body temperature determination and answering questions related to the donor's general health and potential signs of infection or silent bacteraemia, such as the occurrence of recent dental work, or malaise.

Skin Disinfection

Since most PC contaminants are part of the skin flora, optimal skin disinfection of the phlebotomy site is essential to maximise the inactivation of contaminant bacteria during blood donation.

Several factors affect the efficacy of skin disinfection, including the type and concentration of antiseptic used and the mode of application. A two-step method involving a

scrub with a 0.75% povidone–iodine compound *or* a 10% povidone–iodine swab followed by an application of a 10% povidone–iodine preparation solution is outlined in the AABB Technical Manual. A one-step 2% chlorhexidine and 70% isopropyl alcohol skin-cleansing kit has been extensively validated and is being used in the UK, USA, Australia and Canada [17]. A skin disinfection method containing povidone–iodine and alcohol is often used as an alternative for donors allergic to chlorhexidine.

First Aliquot Diversion

Diversion of the first 30–40 mL of blood at the point of collection has been associated with a significant reduction in contamination by skin flora at several blood centres [5,17]. The diverted blood sample is usually used for virology and immunohaematology testing.

Single-Donor Apheresis versus Pooled Platelet Concentrates

An increased risk of bacterial contamination has been traditionally associated with pooled PCs in comparison to single-donor apheresis PCs due to a potential pooling of microorganisms. However, nowadays apheresis donors may donate by a double or triple plateletpheresis procedure and therefore multiple contaminated therapeutic units can be produced, counterbalancing the 'pooling' effect of whole-blood-derived PCs. Studies have shown that the rate of contamination of apheresis PCs and buffy coat platelet pools is similar [6].

Bacterial Detection Methods

Routine testing of PCs for bacterial contamination has been implemented worldwide. Detection of bacteria in transfusable blood components is more complex than viral detection, since bacterial load increases over time under routine blood component storage conditions. Factors that should be considered prior to the implementation of a bacterial screening procedure include the screening method and the testing protocol. Since initial bacterial loads are usually very low (<1 cfu/mL), a very sensitive technique and relatively large sample volume should be used in the blood collection centre shortly after collection. However, less sensitive methods using a smaller sample may be implemented at the hospital end for blood component screening prior to transfusion.

Pretransfusion Detection Methods Used by Blood Centres

The BACT/ALERT 3D and Virtuo automated culture systems (bioMérieux, Marcy l'Etoile, France) and the BACTEC automated culture system (Becton Dickinson, Franklin Lakes, NJ, USA) are the most commonly used methods by blood centres and have been licensed in Europe and North America to detect bacterial contamination in PCs.

The BACT/ALERT and Virtuo systems use liquid aerobic and anaerobic culture bottles with a colorimetric sensor at the bottom that changes colour from green to yellow when pH decreases as a result of the metabolic activity of growing bacteria. The culture bottles are inoculated with 8–10 mL of PC samples and are incubated at 36 °C for up to 7 days, depending on the centre. This system can detect 1–10 cfu/mL of most common platelet contaminants [6]. When an initial positive culture is confirmed by repeat testing of the implicated blood component, a retention sample and/or samples from the recipient, it is considered to be a true (confirmed) positive.

Despite its high sensitivity, several instances of missed bacteria detection in PCs tested by the BACT/ALERT system have been reported worldwide. Examples of microorganisms that have been implicated in false-negative cases include *S. aureus*, *Salmonella*, *S. marcescens*, group A *Streptococcus*, *S. aureus*, coagulase-negative

staphylococci *E. coli, K. pneumoniae, Morganella morganii, P. aeruginosa* and *Acinetobacter* spp., among others [1,2,6–8]. All of these false negatives resulted in septic transfusion reactions. Attempts to decrease the likelihood of false-negative transfusion reactions include increasing the testing volume (often using multiple bottles), delaying sample testing by 24–48 hours after collection, retesting components after 3–4 days of storage, transfusing early during storage or treating products with pathogen reduction technologies, as outlined in the FDA guidance for industry [18].

Most centres routinely test for aerobic bacteria, as most clinically significant organisms belong to this group. Centres using the two-bottle system have reported an increase in the detection rate of bacterial contamination in PCs, since anaerobic culture bottles allow the capture of strict anaerobic bacteria such as *Cutibacterium acnes*. Although cases of transfusion-transmitted *C. acnes* have been reported, none of them has resulted in a severe transfusion reaction. However, there are reports of severe and fatal ATRs due to the presence of the anaerobes *Eubacterium limosum* and *Clostridium perfringens* [2].

The BACTEC system detects bacterial growth in culture bottles based on the production of carbon dioxide (CO_2), which reacts with a fluorescent dye sensor and the system captures the amount of fluorescence produced. Bacteria including *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa* are detected in concentrations ranging from < 1 to 335 CFU/mL. The system is routinely used in some European countries [1].

Other methods developed to detect bacterial contamination in PCs include detection of bacterial 16S rRNA genes by reverse transcriptase polymerase chain reaction (PCR) or PCR-based methods [19].

Bacterial Detection Methods to Be Used Prior to Transfusion

Methods used by the hospital transfusion service can be less sensitive in detecting bacteria in the range of 10^3–10^4 cfu/mL, since bacteria will be of clinical significance only if they have proliferated to these levels during storage, but such tests need to be rapid and specific. The Pan Genera Detection (PGD) and PGDprime immunoassays from Verax Biomedical (Marlborough, MA, USA) and the BacTx colorimetric assay from Immunetics (Boston, MA, USA) have been licensed by the FDA for late PC testing [18]. PC screening with rapid methods is one of the strategies recommended by the FDA to extend the PC shelf-life beyond 5 days.

Pathogen Reduction Technologies

Pathogen reduction technologies involve the treatment of PCs as soon as possible after collection to inactivate or reduce the level of contaminating bacteria, viruses, parasites and residual leucocytes. Three technologies, Mirasol® (TerumoBCT, CO, USA), INTERCEPT™ Blood System (Cerus Europe BV, Amersfoort, The Netherlands) and THERAFLEX UV-Platelets technology (Macopharma, Tourcoing, France), have received CE (Conformité Européenne) Mark registration, while only INTERCEPT has received FDA approval for use in the USA [19,20].

The INTERCEPT process is used within the first 24 hours after collection and utilises a synthetic psoralen, amotosalen HCl, which targets nucleic acid and utilises ultraviolet (UV) A light (3 J/cm^2: 320–400 nm) to form covalent adducts with nucleic acids. INTERCEPT inactivates a broad spectrum of bacterial species associated with transfusion reactions, but cannot inactivate bacterial spores. No septic reactions have been reported in countries where the system has been used for several years [19,20].

The Mirasol system uses riboflavin (vitamin B2, 50 µg per 300 mL) with UVC, UVB and a portion of UVA light

(265–375 nm). The efficacy of this process is based on the association of riboflavin with nucleic acids and the generation of reactive oxygen species, leading to nucleic acid disruption. The efficacy of Mirasol to inactivate bacteria ranges from 33% to 100% [20]. The THERAFLEX system, which utilises UVC light without a photochemical compound, is still in clinical development [19,20].

KEY POINTS

1) Bacterial contamination of blood components poses the most prevalent transfusion-transmitted infectious risk.
2) Platelet concentrates are the blood components most susceptible to bacterial contamination.
3) Interventions such as improved donor screening and skin disinfection, first aliquot diversion, bacterial testing and pathogen reduction technologies have decreased the occurrence of transfusion-associated septic events.
4) Gram-positive skin flora are the predominant blood component contaminants.
5) Gram-negative bacteria are less frequently found as blood component contaminants, but they pose the major infectious risk, causing significant mortality and morbidity in recipients due to their production of endotoxin.

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19 Emerging Infections and Transfusion Safety

Roger Y. Dodd

American Red Cross, Jerome H. Holland Laboratory for the Biomedical Sciences, Rockville, MD, USA

The Institute of Medicine in the USA has defined emerging infections as those whose incidence in humans has increased within the past two decades or threatens to increase in the near future. Emergence may be due to the spread of a new agent, the recognition of an infection that has been present in the population but has gone undetected, or the realisation that an established disease has an infectious origin. Emergence may also be used to describe the reappearance (or re-emergence) of a known infection after a decline in incidence. Some of these emerging infections have properties that permit their transmissibility by blood transfusion; perhaps the most notable example has been human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), although there are others, such as West Nile virus (WNV), dengue virus, Zika virus, *Babesia* and malaria. This chapter will explain the basis for the emergence of infectious agents and discuss their recognition and management in the context of the safety of the blood supply.

Emerging Infections

There is no single reason for the emergence of infections, although it is possible to establish relatively broad groupings [1]:

- Failure of existing control mechanisms, including the appearance of drug-resistant strains, vaccine escape mutants or cessation of vector control accounts for a large group of agents.
- Environmental change can have profound effects, whether through global warming, changes in land utilisation or irrigation practice, urbanisation or even agricultural practices.
- Population movements and rapid transportation can introduce infectious agents into new environments where they may spread rapidly and without constraint, as has been the case for WNV in the US and for SARS-CoV-2 globally.
- Human behaviours can contribute in a number of ways: new agents have been introduced into human populations by contact with, or even preparation and consumption of, wildlife; many infections have been spread widely though extensive sexual networks' and armed conflicts have led to extensive disease spread.

Of course, many of these factors may also work in combination. Key points are that new or unexpected diseases can appear in any location at any time and that an appropriate understanding of the epidemiology of such diseases can assist in the development of appropriate interventions.

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In order to be transmissible by transfusion, an agent must have certain key properties [2]:

- Most importantly, there must be a phase when the agent is present in the blood in the absence of any significant symptoms. Until recently, it was generally thought that such infectivity would reflect a long-term carrier state for the agent in question, as exemplified by HIV, hepatitis B virus or hepatitis C virus, although there had been a few cases of transmission of hepatitis A virus, which provokes an acute infection with a relatively short period of asymptomatic viraemia. However, the finding of transfusion transmission of WNV showed that, in epidemic outbreaks, acute infections could be readily transmitted by transfusion.
- A secondary requirement is that the agent must be able to survive component preparation and storage.
- Finally, the agent should have a clinically apparent outcome in at least a proportion of cases of infection, or it will lack clear relevance to blood safety and its transmission will not generally be recognised. There are some examples of transfusion-transmissible agents that do not seem to cause any significant clinical outcomes, such as GB virus type C/hepatitis G virus (GBV-C/HGV) and torque tenovirus (TTV).

Table 19.1 lists a number of emerging infections that are known, or suspected, to be transfusion transmissible and also notes the factors thought to be responsible for their emergence.

Approaches to the Management of Transfusion-Transmissible Emerging Infections

As far as possible, emerging infections that do, or may, impact on blood safety should be managed in a systematic fashion. In general, this will be the responsibility of agencies that are charged with the maintenance of public health, or the management of the blood supply or its regulation. However, there are several areas in which individual professionals can contribute. One of these is the first step, which is the recognition of a transfusion-transmitted infection and its subsequent investigation. It is, in fact, unlikely that the first occurrence of an emerging infection will be seen in a transfused recipient, so it is therefore important that there be a system of assessing the threat and risk of emerging infections for their potential impact on blood safety. This implies a process for evaluating each emerging infection for its transmissibility by this route and for estimating the severity and potential extent of the threat. The risk assessment should help to define the need for and urgency of development and implementation of interventions to reduce the risk of transmission of the agent. Such interventions, if implemented, should be evaluated for efficacy and modified as appropriate.

Increasingly, at least in the USA, there is declining use of blood and financial pressures on hospitals. This appears to be driving decisions on the adoption of blood safety issues towards the hospitals and away from the blood providers. In these circumstances, practitioners responsible for hospital blood services may find that they need to advocate for safety improvements within their hospital's management structure.

Assessing the Risk and Threat of Transfusion Transmissibility

It is important to have a general awareness of the status of new and emerging infections, with particular reference to your own country or area. Such an awareness may involve familiarity with a number of sources of information, ranging from news media, through to alerts from local, national and global public health agencies, to specialised resources such as ProMED Mail (an internet list server and website that tracks and comments on disease outbreaks) [3]. Other tools continue

Table 19.1 Selected emerging infections potentially or actually transmissible by blood transfusion.

Agent	Basis for emergence	Notes
Prions		
vCJD	Agricultural practice: feeding meat and bonemeal to cattle	Of most concern in the UK; apparently coming under control
Viruses		
Chikungunya	Global climate change, dispersion of mosquito vector, travel	Rapid emergence in a number of areas, including Italy, the Caribbean and the Americas. Surveillance indicated
Dengue	Global climate change, dispersion of mosquito vector, travel	Similar properties to WNV; surveillance indicated, limited testing implemented
HBV variants	Selection pressure resulting from vaccination	Mutants may escape detection by standard test methods
HHV-8	Transmission between men who have sex with men and perhaps by intravenous drug use	Transmission by transfusion and transplantation known
HIV	Interactions with wildlife, sexual networks, travel	Classic example of an emerging infection
HIV variants	Viral mutation, travel	May escape detection by standard tests
Influenza	Pandemic anticipated as a result of antigenic change	Possible threat to blood safety, major impact on availability
SARS and SARS-CoV-2	Explosive global epidemic, wildlife origin, spread by travel	No demonstrated transfusion transmission, epidemic
Simian foamy virus	Exposure to monkeys, concern about species jumping and mutation	Regulatory concern over blood safety, intervention in Canada
WNV	Introduction into the USA (probably via jet transport), rapid spread across continent	Recognition of transfusion transmission in 2002 led to rapid implementation of NAT for donors
Zika virus	Introduced to Brazil via air transport	Explosive expansion through Americas via *Aedes* spp.; major concern over causation of microcephaly, rapid implementation of multiple interventions
Bacteria		
Anaplasma phagocytophilum	Tick-borne agent expanding its geographicall range	At least 8 potential transfusion transmissions reported
Borrelia burgdorferi	Tick-borne agent expanding its geographicall range and human exposure	No transfusion transmission reported
Coxiella burnetii	Intensive goat farming in The Netherlands, human exposure	Presumptive evidence of transfusion transmission, donor selection and NAT implemented
Parasites		
Babesia spp.	Tick-borne agent expanding its geographicall range and human exposure	More than 200 transfusion transmission cases reported Geographically selective investigational testing

(*Continued*)

Table 19.1 (Continued)

Agent	Basis for emergence	Notes
Leishmania spp.	Increased exposure to military and others in Iraq, Afghanistan	Unexpected visceral forms potentially transmissible
Plasmodium spp.	Classic re-emergence, in part due to climate change, travel	Re-emergence threatens value of travel deferral
Trypanosoma cruzi	Imported into non-endemic areas by population movement	Transfusion transmissible, preventable by donor testing

HBV, hepatitis B virus; HHV, human herpes virus; HIV, human immunodeficiency virus; NAT, nucleic acid testing; SARS, severe acute respiratory syndrome; SARS-CoV-2, COVID-19 virus; vCJD, variant Creutzfeldt–Jakob disease; WNV, West Nile virus.

Box 19.1 Key questions to assess risk of transfusion transmissibility of an infectious agent

1) Have transfusion-transmitted cases been observed?
2) Does the agent have an asymptomatic, blood-borne phase?
3) Does the agent survive component preparation and storage?
4) Are blood recipients susceptible to infection with the agent?
5) Does the agent cause disease, particularly in blood recipients?
6) What are the severity, mortality and treatability of the disease?
7) Are there recipient conditions, such as immunosuppression, that favour more severe disease?
8) Is there a meaningful frequency of infectivity in the potential donor population?
9) Is this frequency declining, stable or increasing?
10) Are there reasons to anticipate any changes in the frequency of donor infectivity?
11) What is the level of concern about the agent and its disease among professionals, public health experts, regulators, politicians, media and the general population?
12) Are there rational and accessible interventions to eliminate or reduce transmission by transfusion and to manage other impacts on transfusion medicine?

to become available; for example, the Association for the Advancement of Blood and Biotherapies (AABB) maintains a listing of potentially transfusion-transmissible infectious agents that has been published in print and on its website; the listing also contains much of the information discussed below, along with a ranking of the threat level. Other agencies (for example the Centers for Disease Control and Prevention and the World Health Organization) provide general, current information about emerging infectious agents on their websites.

Box 19.1 outlines questions that serve to define the risk of transfusion transmission of each agent and the potential extent and severity of that risk. The primary question is whether or not the disease agent can, in fact, be transmitted by blood. As pointed out above, this is dependent on the presence of an asymptomatic phase during which the disease agent is present in the bloodstream. In some cases, of course, there may already be documentation of transfusion transmission of the agent in question, or there may be suggestive evidence, such as transmission by organ transplantation. However, in the latter case such evidence may not be definitive, as rabies has been transmitted by organ transplantation but is almost certainly not

transmissible by blood. The answer to this question is not always available, but may often be inferred by considering what is known about the natural transmission route of the infection, or from the properties of closely related organisms. The duration of the blood phase of the infection will have a direct impact on the risk of transmission, reflecting the chance that an individual will give blood during the infectious phase.

The actual risk of transmission is a function of the frequency of the infection in the donor population and the length of the period of blood-borne infectivity [4]. The period of infectivity may not, however, be identical to the period during which the infectious agent can be detected in the blood. For example, in the case of WNV, periods of viraemia in excess of 100 days have been measured occasionally, but the actual infectious period may be limited to the week or two prior to the appearance of immunoglobulin (Ig)G antibodies. Another difficulty is that the frequency of disease and the frequency of infection may differ greatly, as is again the case with WNV.

Nevertheless, it is abundantly clear that individuals who do not develop symptoms may be infectious via their blood donations. Consequently, it may be important to estimate the size of the infected (and infectious) population by laboratory testing rather than through disease reporting. Indeed, organised studies of prevalence rates of infection among donor populations have been used in many circumstances in order to assess the level of risk and to predict the impact of a testing intervention. Examples of this approach include studies on human T-lymphotropic virus (HTLV), trypanosomes (*T. cruzi*), Babesia and, more recently, dengue virus, where assessments of the frequency of viraemia are proving valuable.

Another important factor is the dynamics of the outbreak. Is the frequency of infection stable or increasing, and if increasing, is change linear or logarithmic and what is the rate of increase? Obviously, rapid increase, as seen in the case of WNV, would imply a need for a more rapid response than would a slow, linear increase, as in the case of *T. cruzi*.

The severity of disease that may result from a transfusion-transmitted infection is also an important guide to the extent and speed of implementation of any intervention. There are both objective and subjective aspects to such an assessment. Clearly, the severity of the disease and its associated mortality can be defined, but it may also be important to judge the public concern around the disease, which may be disproportionate to its actual public health impact [2]. Another factor that is often presented as important is the extent to which a transfusion-transmitted infection might result in further or secondary infections. In actual fact, transmission of an infection by transfusion will almost certainly not lead to any magnification of an epidemic, but nevertheless, it is something that should be considered.

A word of caution is in order with respect to efforts to use modern laboratory methods to identify previously unrecognised infectious agents. There is increasing enthusiasm for this approach, but it is important to recognise that without any established relationship to a disease state, the results of such searches can be misleading. At this time, for example, it does not appear that either TTV or GBV-C/HGV has any relationship to any disease state, and they do not seem to offer risk to blood recipients, despite clear evidence of their transmissibility. It is unclear how many other such orphan viruses are awaiting discovery.

The recent recognition and management of a new retrovirus, xenotropic murine leukaemia virus-related virus (XMRV), originally thought to be associated with prostate cancer and chronic fatigue syndrome (CFS), is instructive. It was suggested that this virus was a threat to transfusion safety and an organised programme was put in place to evaluate that possibility [5,6]. A complication was that CFS advocates actively promoted the concept of transfusion risk to establish legitimacy (and perhaps funding) for the

disease. A key activity was a careful, blinded evaluation of many different tests for XMRV, including those used by the laboratories responsible for the original discoveries. This evaluation, along with other studies, revealed that the available tests could not reliably identify the virus or related ones, in either patient samples or negative controls [7]. The original observations were eventually shown to be due to various forms of contamination and XMRV itself was revealed to be a laboratory artefact. While early intervention for an emerging infection may be necessary and appropriate, care should be taken to avoid reacting to situations involving incomplete or imperfect science.

Recognition of Transfusion Transmission of Emerging Infections

There is no simple formula for recognising that a transfusion-transmitted infection has occurred, particularly in the case of a rare or unusual disease agent. Nevertheless, many such events have been recognised by astute clinicians. Knowledge of the potential for transmission of an emerging infection can be valuable and very likely contributed to the relatively early recognition of transfusion transmission of WNV [8]. Unusual post-transfusion events with a suspected infectious origin should be brought to the attention of experts in infectious diseases or public health agencies for assistance in identification and follow-up. Appropriate investigation of illness occurring a few days or more after transfusion can reveal infections through identification of serological or molecular evidence of infectious agents in posttransfusion samples. However, such detection is by no means definitive. It is helpful if a pretransfusion patient sample is also available, as this will reveal whether the condition predated the transfusion. Also, recall and further testing of implicated donors will reveal whether one or more of them was the likely source of the infection. Ideally, if the responsible organism can be isolated from both donor and recipient, molecular analyses such as nucleic acid sequencing can demonstrate (or exclude) the identity of the agent from the two sources.

Information from the donor may also be a critical source of identification of a transfusion transmission. More specifically, donors are requested to report to the blood collection facility the occurrence of any signs of illness in the few days after donation. This allows the blood components to be recalled or, if already transfused, should lead to investigation of the recipient. The first two reported cases of transfusion-transmitted dengue were identified in this fashion. Similarly, lookback to recipients of prior donations from a donor with a newly recognised infection may identify transmission of a chronic infection. Indeed, all cases of transfusion transmission of HIV in the USA since 1999 were recognised through lookback.

There are significant problems in recognising that infections with a very long incubation period may have been transmitted by transfusion; this was illustrated by HIV/AIDS, which did not result in well-defined illness until many years after exposure. That prevented early recognition of transfusion-transmitted AIDS and further concealed the actual magnitude of the infectious donor population and of the population of infected blood recipients. This implies that, for emerging infections that appear to have lengthy incubation periods, it would be wise to assess transfusion transmissibility by serological or molecular evaluation of appropriate donor–recipient sample repositories, or to engage in some form of active surveillance such as that used to identify the transmission of variant Creutzfeldt–Jakob disease (vCJD) by transfusion in England [9]. Haemovigilance programmes may contribute to the identification of post-transfusion infections, although they are generally designed to identify well-described outcomes.

Interventions

If an emerging infection is found to be transfusion transmissible and public and professional concern implies a need to protect the safety of the blood supply, there are a number of interventions that could be considered.

A possible but rather unsatisfactory approach is to focus on the recipient by diagnosing and treating cases that occur. This, of course, works only for treatable infections. It was *de facto* part of the approach to manage transfusion babesiosis in the USA prior to implementation of regional donor testing. In the case of highly localised outbreaks, or where there are alternate sources of blood, it may be possible to stop collection of blood in the affected area.

Most interventions are focused on the donor or the donation. In the absence of a test, it may be possible to devise a question that would identify some proportion of donors at risk of transmitting the infection. Such measures are usually neither sensitive nor specific but may have value, particularly where the disease is localised so that a travel history is sufficient to identify those at risk.

The development and implementation of a test for infectivity in donor blood usually constitute a more sensitive and specific approach than questioning, and for some infections may be the only valid solution. In the past serological tests were relied upon, but now nucleic acid testing (NAT) is also available and may be a better solution, as was the case for WNV. Indeed, a test for WNV RNA was developed and implemented in less than a year in the USA [10]. However, this is not always the optimal solution. For example, some parasitic diseases in particular result in long-term, antibody-positive infection with very low levels of infectious agent in the bloodstream, resulting in only intermittent NAT-positive findings. This is particularly true of Chagas' disease, and as most individuals were infected early in life, antibody tests are preferable for identifying potentially infectious donors [11].

An emerging technology that offers some promise is pathogen reduction, which is a treatment that inactivates infectious agents in blood while retaining the biological activities of the blood itself. Methods are currently available for plasma and for platelet concentrates and are in use in some countries. It should be noted that available methods may have differing efficacies for different infectious agents and that they may not be fully successful in eliminating very high levels of infectivity for some agents, although this has not been established in practice. A real disadvantage is that no method is currently available for red cells. A pathogen reduction method was implemented for platelets in the island of La Réunion during a large outbreak of chikungunya virus infection and has been used for recent Zika outbreaks.

The precautionary principle is often cited when interventions to reduce the risk of transfusion-transmitted infections are discussed. In general, it is suggested that, in the absence of any specific information about the efficacy of an intervention, it is appropriate to implement it, as long as it does no harm. This position may be arguable, particularly as commentary on the precautionary principle suggests that it should not be invoked without some evaluation to ensure that the measure is not extreme and does not exceed other measures taken in known circumstances. In fact, significant measures were taken to reduce the potential risk of transmission of vCJD even before it was known that it was transmissible by transfusion. It can be argued that subsequent events justified the precautions taken, but this may not always be the case [12].

Pandemic Preparedness

The emergence of a novel coronavirus (SARS-CoV-2) in China, and its rapid expansion to pandemic status, with more than 100 million recorded cases and 2.2 million

deaths worldwide by January 2021, has been a salutary experience. Most countries have suffered from inadequate responses, although in a few cases exemplary action has essentially eliminated risk. Early experience and comparison with prior coronavirus outbreaks strongly suggested that SARS-CoV-2 was not transmissible by transfusion, so direct measures to prevent such transmission were not necessary [13]. However, the pandemic has had a profound impact upon the availability of blood. Mechanisms to ensure the safety of donors and of staff members were essential. In addition, measures had to be taken to reassure donors and to encourage their continual commitment. Extensive efforts were also undertaken to collect convalescent plasma as a potential treatment or prophylactic measure, but at the time of writing the efficacy of this measure is unclear.

The lesson is that pandemic preparedness is a critical component of the management of transfusion medicine. In this context, organisations that had such policies, developed as a response to earlier threats of influenza pandemics, were better able to respond to the threat of COVID-19. It is abundantly clear that, now and for the future, pandemic planning will be critical at all levels of transfusion medicine.

Making decisions about the implementation of blood safety measures is very complex and requires consideration of many objective and subjective factors. Increasingly, attention is being focused upon the process of risk-based decision making, which has recently been formalised [14].

KEY POINTS

1) Some emerging infections may threaten the safety of the blood supply.
2) Those responsible for maintaining the safety of the blood supply should be familiar with emerging infections.
3) Physicians responsible for the care of transfused patients should be alert for signs of unexpected infections.
4) The nature and extent of the safety threat offered by emerging infections may be assessed by examination of a fairly simple sequence of questions.
5) If interventions are needed, consideration should be given to the use of donor questions and/or laboratory tests.
6) Care must be taken to balance public concern against good science.

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20 Regulatory Aspects of Blood Transfusion

Louis M. Katz[2], Peter Flanagan[3] and William G. Murphy[1]

[1] *Irish Blood Transfusion Service (retired); University College Dublin, Dublin, Republic of Ireland*
[2] *ImpactLife Blood Services, Davenport, IA; Infectious Diseases, Carver College of Medicine, University of Iowa Healthcare, Iowa City, IA, USA*
[3] *New Zealand Blood Service, Wellington, New Zealand*

Blood is an irreplaceable medicine used in life-saving circumstances. Every country's government must ensure that its citizens and visitors have access to an adequate supply of safe and effective blood for transfusion. The World Health Organization (WHO) has identified the importance of a well-legislated and regulated blood transfusion service as a crucial component in assuring safety [1]. Because it is necessary to source blood from biologically and behaviourally heterogeneous humans, absolute uniformity of product and its absolute safety cannot be guaranteed, no matter how extensively we interrogate donors, and test and process their donations. Pragmatic compromises must be reached between adequate safety and adequate supply.

Government, through the regulatory agencies, has explicit responsibility for overseeing the safety of blood for transfusion. Blood suppliers also have an ethical and professional responsibility to go beyond the minimum regulatory or legal requirements in the interests of safety if the regulator does not require sufficient quality, and in any event, they have a responsibility to ensure that standards are met in a consistent manner. Government is also ultimately responsible for the adequacy of supply and must ensure that an appropriate compromise between safety and supply is achieved.

The Components of Blood Regulation

Guiding Principles and Law

National or international law, and which is defined and enacted by legitimate representatives of the society concerned, establishes the regulatory framework for blood components in a jurisdiction. A competent system of regulation must be backed by such law, which specifies and limits the scope and power of the regulator. The law should be explicit, and production of blood for transfusion in a state should be expressly governed by statute. For example, within the European Union (EU) member states, the national statutes are subject to the Blood Directives – a set of laws developed over several years between 1996 and the present that are binding on all the member states of the Union. There is a core or parent Directive [2] and a set of subsidiary

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ones covering donors and donations and component specifications [3], traceability and haemovigilance [4] and quality systems [5,6]. Additional updates and modifications may appear in response to emerging threats or technical advances. Elsewhere national, federal, provincial and state laws almost invariably provide a basis for regulation, though in several parts of the world such laws and regulations may be merely aspirations [7].

Guiding principles may be explicit or implicit. For example, the preamble to the core Directive [2] contains statements of principle, scope and intent that have a legal function in the event of dispute. It states, for example, that the intent of the regulations is to ensure public health, thereby forever giving blood safety in the EU primacy over legitimate commercial interests in the drafting and execution of regulations. The EU Quality Directive identifies the need for 'Principles of Good Practice' as the principles covering quality systems in blood establishments (Article 2 of 2005/62/EC [5]). These became available only in 2016 with the publication of the Good Practice Guidelines [8] and introduced as requirements by the accompanying Directive 2016/1214/EC [6]. The Therapeutics Goods Administration (TGA) in Australia produces a specific Code of Good Manufacturing Practice (GMP) for blood and tissues. This includes both quality and technical standards that must be met. The US Food and Drug Administration (FDA) states that GMPs are the guiding principles in the functioning of blood services. GMPs are a dynamic and very comprehensive set of overall directions, principles, rules and regulations that reduce the entropy in a complex system, and thereby the likelihood of mishap. Regulators, however, draft regulation as well as enforce it, and can and do increase complexity beyond any possible meaningful improvement in safety. This requires a constant dynamic in the regulatory process in the light of emerging knowledge, science and understanding; see below.

The precautionary principle emerged in the 1980s as a statement of best practice when faced with serious but unquantifiable risks. This principle was enshrined at the 1992 Rio Conference on the Environment and Development: 'Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.' Since then, it has come to be explicitly adopted for health protection, for example in the EU [9] and in Canada [6,10]. It continues to develop and evolve and to gather increased force of law through case law in Europe and elsewhere.

Most formulations of the precautionary principle include explicit acknowledgement that decisions taken under its aegis are subject to reconsideration and modification as more complete data are accrued. This results in tensions over what constitutes sufficient evidence to undertake amendment of long-standing regulatory equipoise between risk and benefit.

Standards

Professional advisory bodies as well as technical standards bodies such as the International Standards Organization (ISO) may set product and process standards to be adhered to for accreditation, whereas regulators define minimum rules and regulations with which blood component and service providers must comply to operate within the law. Regulators may, however, mandate accreditation by other bodies as a regulation to be complied with. In this way, standards of the ISO, national technical standards bodies or professional bodies such as the Association for the Advancement of Blood and Biotherapies (AABB) and the College of American Pathologists (CAP) in the USA may be explicitly applied to blood component or transfusion service providers and may be given force of law. Standards used for accreditation and regulatory purposes need to be current; they also need an explicit mechanism of review and change as scientific knowledge accrues, circumstances change and experience informs.

Application and Enforcement of Regulations

A regulation is of limited value if it is not or cannot be enforced because the rule of law is lax, the enforcing agency is weak or under-resourced or the standard is unrealistic or unrealistically expensive. There are three levels of application of regulations and standards in the regulatory setting.

Inspection/Accreditation/Licensing

In most countries with a robust health system, all bodies participating in the business of providing blood for transfusion are required to be accountable through an official process, usually by some combination of registration, accreditation and licensing. Self-inspection, or accreditation by peer review, is generally no longer considered acceptable in isolation. The scales of licensing requirements that apply to facilities that collect and test blood from donors are often different than those for hospital blood banks, who either store blood for transfusion and issue it, or conduct minimal processing. Licences are time limited and require renewal, with reinspection typically every 1–3 years.

Enforcement

The rule of law demands compliance. Regulatory authorities can and do apply fines, sometimes of millions of dollars, to blood establishments that fail to comply with legal requirements. Hospital blood banks may be closed for serious quality failures. Blood transfusion staff may be arrested and tried in a court of law, though usually only as a result of egregious events rather than for systematic failures detected through routine application of regulatory methods.

Threat Surveillance

Some regulatory agencies have adopted a formal threat surveillance role, by which they generally mean infectious disease threat surveillance. For example, the FDA works closely with blood establishments, the Centers for Disease Control and other agencies in the Department of Health and Human Services in the USA, and in Europe the European Commission works with the European Centre for Disease Control. Haemovigilance systems augment this approach. The Alliance of Blood Operators (ABO) has developed a risk-based decision-making framework to help address emerging threats in a systematic manner [11].

Vigilance: Haemovigilance/Feedback/Market Surveillance

Large-scale, systematic, well-organised surveys of clinical outcomes over tens or hundreds of thousands of recipients are required to track the occurrence and even to establish the existence of problems with the function, safety and quality of medicines, devices or processes. Within blood transfusion practice, this is known as haemovigilance. Originally mandated in France and voluntary in the UK, it has evolved to become mandatory in many jurisdictions. In the USA, a formal haemovigilance initiative was integral to the modification of blood donor deferrals for male-to-male sexual behaviours [12]. In the EU, haemovigilance also extends to adverse effects in blood donors. This will change approaches to donor care as more valid statistics on the incidence of very rare events emerge, and lead to mandatory approaches to such common events as iron deficiency.

The Regulatory Bodies

There are several types of bodies involved in the regulatory framework or structure in blood transfusion (Table 20.1). They contribute to the formulation or application of the law and/or the formulation, application or review of standards, or they can enforce the regulations.

Table 20.1 Agencies involved in regulatory processes in blood transfusion.

Type of agency	Name	Role	Further information
Multinational agency with statutory powers	European Commission	Drafts laws for implementation in European Union (EU) member states. These laws include technical specifications for blood components, for example. Oversees the application of those laws through national authorities – the 'competent authorities'	Lacks a well-defined structure for scientific analysis of the impact of the laws on healthcare and health economic implications of practices; has a limited but developing ability to adapt to advances in the field
National agency under statutory authority from legislative bodies with regulatory powers, and significant scientific/ developmental/leadership roles	Food and Drug Administration (FDA; USA); Paul Ehrlich Institute (PEI; Germany); Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM; France)	Defines national rules and regulations and licenses the blood establishments in national or federal setting. Conducts scientific programmes aimed at developing the field of blood banking and the scientific basis of regulation, and publishes its work in a peer-reviewed environment	Within the EU, the member states have to comply with the basic requirements of EU laws; beyond that they are free to impose other requirements and standards. Germany, through the PEI, licenses blood components as medicines, as does Austria. In the USA, the FDA establishes minimum requirements for safety, purity and potency and licenses and inspects facilities against them and maintains an active research programme in support of good regulation and guidance
National agency under statutory authority from legislative bodies with regulatory powers, but without significant in-house scientific activity, though in some cases they provide scientific leadership through structured partnerships between the regulator, blood transfusion service and government agencies	Health Canada, the Therapeutic Goods Administration (Australia), Medicines and Healthcare products Regulatory Agency (MHRA; UK), South Africa, New Zealand, EU member states, Brazil, etc.	Inspects and licenses blood banks and blood transfusion services on its territory in accordance with national laws (in the EU these national laws are subservient to EU law and must include all the EU requirements as a minimum)	
National agency that also manages the blood supply	In several of the former Communist countries, for example, a combined central power base was established at the national blood service provider, who then assumed the role of regulator in the EU setting	Both provider and regulator. Very difficult to avoid the impression of failure of separation of responsibilities, i.e. a conflict of interest	

(*Continued*)

Table 20.1 (Continued)

Type of agency	Name	Role	Further information
International treaty-defined agency with considerable leverage in national and international blood banking policies and regulations	World Health Organization (WHO)/Pan-American Health Organization (PAHO)	The WHO requires member states to develop policies and standards in national blood transfusion systems	The WHO carries considerable force in many parts of the world, but much less so in high development index areas. In several countries dependent on aid for blood transfusion development, the WHO approach has quasi-regulatory status
	Council of Europe* (European Directorate for the Quality of Medicines and Health Care, EDQM)	The Council of Europe has for many years produced an annual technical guideline, which attempted to raise the bar of quality in member states in an iterative fashion. This function has now devolved to the EDQM under the influence of the European Commission. The Guide is now published every two to three years. Recent editions have begun to separate standards (requirements) from principles (recommendations). Increasingly EDQM is aiming to position itself as the primary organisation managing the technical aspects of the Directives. This in many ways mirrors its work in managing the European Pharmacopoeia	This situation is in evolution – the EDQM is developing a more formal link with the European Commission in this function, i.e. providing the technical expertise the Commission needs to maintain regulation in blood transfusion (the only country to date to give the Council of Europe Guide regulatory force is Australia)
Supranational incorporated bodies that have no statutory powers, but seek to provide scientific leadership and define standards of professional practice	Association for the Advancement of Blood and Biotherapies (AABB), International Society of Blood Transfusion (ISBT)		

* The Council of Europe is not the European Council – the former is a union to promote humanitarian values and principles in law, rights and health, among other areas, and produces advice and works by recommendation and peer pressure between states; the latter is an administrative arm of the EU. (The other two administrative arms are the Commission – essentially a very powerful civil service – and the Parliament. While all EU member states belong to the Council of Europe, not all Council of Europe member states belong to the EU.)

National Statutory Bodies with Powers of Enforcement

Most high and medium development index countries have a functioning national medicines agency that sets and applies standards for drugs and biologics being manufactured, imported, sold and prescribed in that country. Similar bodies, in many cases the same body, set and apply standards for labile blood components. They have the force of law and can fine or imprison those who break the law. A person setting up their own blood collection service within the EU, for example, without a licence from the relevant government agency would face serious charges. Examples of such bodies are the FDA in the USA, Medicines and Healthcare products Regulatory Agency (MHRA) in the UK, Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) in France, Paul Erhlich Institute (PEI) in Germany, TGA in Australia, Health Canada in Canada and the various national agencies in the EU and other European countries. They are not all the same: some, notably the FDA, PEI and ANSM, have developed roles as leaders in blood transfusion science and participate actively in developments in the field. In several countries, including some of the old Eastern bloc members of the EU, the agency within the state responsible for regulation (in the EU these agencies are called 'competent authorities') is also the national transfusion service, reflecting older Communist structures. In these cases, it is very difficult to avoid the perception of conflicting interests.

Supranational Agencies with Statutory Powers

The European Commission in the EU – essentially the civil service of the European Union – has very broad powers in the field of consumer safety and citizens' health, including explicit powers under the founding treaty for ensuring blood safety. A series of laws (Directives) have been enacted since 2002 that cover licensing of blood establishments, accreditation of hospital blood banks and technical specifications, from donor qualification to component transportation. In addition, the Directives address traceability and haemovigilance. The Commission has very little in-house expertise in blood transfusion and uses industry expertise, sourced mainly through the Departments of Health in the member states, to provide support.

The FDA in the USA is of a similar scale and scope, insofar as it has a remit across the 50 states, a number of territories and commonwealths, and the District of Columbia. The USA carries substantial weight in the industry far beyond other national statutory agencies. It has a very mature and heavily resourced function in defining standards and direction of research and, uniquely, decides which tests or other technologies must and may be applied within its jurisdiction.

Supranational Agencies with Treaty-Defined Powers and Remits

There are two of these: the World Health Organization (WHO; including the Pan-American Health Organization, PAHO) and the Council of Europe. They may exclude countries from membership, but in reality their power to enforce rules is limited and their function is essentially advisory, although they can provide support with funding or with channelling of funding from others. While the WHO's involvement in blood transfusion carries considerable weight in medium and low development index countries, this is not the case in high index ones. The WHO's approach is essentially to promote national policies around a central nationally coordinated blood transfusion service with accountability to government.

Professional Organisations within the Blood Transfusion Community Itself That Set or Define Professional Standards

These bodies have no statutory powers. Examples include the AABB and the International Society of Blood Transfusion (ISBT), and national professional organisations.

They have an important regulatory function in providing professional support, including defined professional standards.

Stakeholder Input into Regulatory Decision Making

The regulatory agency is responsible, on behalf of the government, to maintain an effective regulatory system and to monitor, and where appropriate enforce, compliance. Many organisations will wish to have an ability to influence the decisions and processes of the regulatory authority. This stakeholder input to decision making is an important mechanism to ensure community support and engagement. A range of organisations from industry will play an important advocacy role along with consumer organisations representing both patient and donor interests. Regulatory agencies must ensure that effective systems are in place to make sure that this stakeholder input occurs.

There are many international and national organisations that bring important knowledge and skills to the decision-making process. These include the International League of Red Cross and Red Crescent Societies, America's Blood Centers (ABC), the European Blood Alliance (EBA), the Alliance of Blood Operators (ABO) and professional trade associations such as the International Plasma Producers Congress (IPPC), the Plasma Protein Therapeutics Association (PPTA) and the International Plasma Fractionation Association (IPFA).

The voice of the patient in regulation of blood transfusion, where expressed, is generally through the political process and the governance arrangements for regulators, hospitals and blood suppliers. In addition, several well-organised groups representing users of blood components – people with thalassaemia and sickle cell anaemia – and plasma products – people with haemophilia or primary immune deficiencies – have valid concerns and often provide formal input into regulatory processes [13]. The patient organisations can be very powerful campaigners for change in the legislation or regulation of blood components, especially where supply and safety may be compromised.

Donor associations exist as separate entities to blood transfusion services in several countries and have an international body, the International Federation of Blood Donor Associations. From time to time, these groups may lobby regulators, governments and blood suppliers. Many such organisations actively promote voluntary unpaid donation, and are strong supporters of blood collection programmes. Their influence and activity vary considerably from country to country

Public bodies, for example lesbian, gay, bisexual and transgender and allied advocacy (LGBTA+) groups, may become involved in public debates and issues surrounding blood transfusion from time to time. In particular, they aim to address perceived inequalities and discrimination in donor deferral policies or in access to services and products. This approach has been very successful in relaxing restrictions on men who have sex with other men in several jurisdictions in recent years.

Effective systems for stakeholder input mean that virtually any individual or *ad hoc* interest group can lobby regulatory agencies and standard-setting organisations at will.

The Role of Blood Transfusion Agencies and Health Professionals vis-à-vis Regulatory Agencies

Laws, regulations, ethics and social values evolve. What is accepted as ethical and proper in one time and place may not be legal in another. Laws governing gender and sexual identity are but one example. So, while the law as it is must be observed and respected, it need not be revered as complete, correct or immutable. Neither should it be seen to be outside the sphere of influence of the public to whom it applies. In contrast, it is the civic duty of citizens of a state and the ethical duty of

scientists and physicians everywhere to attempt to correct deficits or address shortcomings in the law, whether through contributing to the drafting of new statutes, correcting technical errors, including omissions, in the law itself or advocating repeal when the prevailing conditions render laws obsolete. This must be effected through legal and transparent processes, perhaps ideally through competent and legal organisations for this purpose, including, in blood transfusion, the AABB, ABC, EBA and similar bodies.

In almost no country are the regulatory authorities concerned with improvements in the efficacy of red cells or platelets, or with treatment protocols and guidelines. These remain the preserve of the professionals in the field. Blood remains a problematic medicine; regulation tends by its nature to be very conservative and to preserve the status quo at the expense of development. This may give rise to a natural and often constructive tension between regulators and practitioners, to whom it is obvious that there is much to improve. The development of high-quality clinical and laboratory evidence to support a change in the regulations is crucial.

The COVID-19 pandemic has required urgent and emergent responses in both the blood community and by regulators worldwide. These have included development and control of the collection and distribution of biologics for passive immune therapies (i.e. convalescent plasma, monoclonal antibodies and hyperimmune globulin). This has been in the face of inadequate data from high-quality clinical trials and under political pressures. While that is beyond the scope of this chapter, the reader is referred to exemplary regulatory documents and commentary to judge the quality and pitfalls of this emergency decision making [13–15].

KEY POINTS

1) A well-legislated and regulated blood transfusion service is a crucial component in assuring safety of the blood supply within a country or state, although there are large variations among different jurisdictions in how this is addressed.
2) Regulations should, but do not always, address issues of adequacy of supply and availability of blood within a state, as well as quality, safety and scientific developments.
3) Regulations are based in law, which should be explicit in statute, and are governed by guiding principles, often in practice a version of the precautionary principle and the principles and rules of good manufacturing practice.
4) State regulatory agencies apply standards; these are often set by national accreditation or standards bodies, or by professional bodies within the field.
5) The nature and scope of regulations may be influenced by forces inside or outside blood transfusion: lobbying by professional groups, trade organisations, patients and other interested groups and bodies can apply pressure for change at the political and public levels.
6) Regulatory compliance is always part of professional and scientific integrity and endeavour, but it is never all of it, much less a substitute for it.

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Further Reading

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21 The Role of Hemovigilance in Transfusion Safety

Katharine A. Downes[1] *and Barbee I. Whitaker*[2,*]

[1] *University Hospitals Cleveland Medical Center and Case Western Reserve University, Cleveland, OH, USA*
[2] *Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA*

Haemovigilance is a set of surveillance procedures designed to improve the safety and outcomes of transfusion recipients and blood donors through the collection and assessment of information about unexpected and/or undesirable effects resulting from the donation of blood and the therapeutic use of labile blood products, and to prevent their occurrence or recurrence [1]. Development and implementation of haemovigilance systems began nearly 30 years ago, driven initially by the emerging infectious risks of human immunodeficiency virus (HIV) and hepatitis to blood safety. They have since evolved to evaluate the safety throughout the transfusion chain, from blood collection to blood administration and its subsequent outcomes [2]. Such systems facilitate understanding of transfusion safety risks within the transfusion chain, including differences in practice and the identification of steps in the process that are associated with superior outcomes. These early, national systems have led to a global haemovigilance movement focused on blood safety, learning from one's practice and a commitment to continuous improvement, resulting in changes to the practice of blood collection and transfusion therapy.

Haemovigilance System Organisational Structures

Haemovigilance systems may be found throughout the world, with organisational, regulatory, legal and reporting practices that vary by country. The European Union requires implementation of a haemovigilance system in each member state, with reporting to a central office [3]. Haemovigilance functions may reside within and derive reporting mandates from a national ministry of health, a regulatory authority, a public health agency, or be organised collaboratively through professional societies or the national blood collection system or through public–private partnership [4]. While each country has developed characteristics unique to its own healthcare ecosystem, haemovigilance systems bear multiple similarities and ideally yield similar results [5]. Key features of successful haemovigilance systems are listed in Table 21.1.

Haemovigilance system reporting requirements may be voluntary or mandatory. A non-punitive approach to reporting – for the reporting individual and the institution – is essential for compliance. The degree to which an institution focuses on outcome improvement and adherence to policies is probably an

*Disclaimer: Dr Whitaker's contributions are an informal communication and represent her own best judgement. These comments do not bind or obligate FDA.

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Table 21.1 Key features of a haemovigilance system.

Confidentiality of submitted data
Broad participation, supported by education
Standardised definitions and terminology
Non-punitive data evaluation
Reporting of occurrences using rates
Sufficient detail to make effective recommendations to improve practice(s)
Focus on improved safety and outcomes
Simple, efficient operations
Sustainable organisation
Timely and transparent release of analysis findings

important determinant of haemovigilance system acceptance and high rates of reporting compliance. An approach to looking at unexpected adverse events from a system perspective rather than at the individual(s) involved with the event may make it more likely that staff will feel comfortable reporting occurrences when 'incidents' are considered system failures rather than failures of personnel. Even with confidentiality safeguards, voluntary reporting is unlikely to attract haemovigilance participants unless the value of reporting is understood. System endorsement by professional associations or the ministry of health may boost participation in a voluntary reporting system, particularly if the system is easy to use.

Whether a system is voluntary or mandatory, local preparation is a key to successful participation and results. Effective preparation includes conducting a detailed review of current processes and procedures for adverse reaction reporting and a gap analysis compared to what will be expected to participate in the haemovigilance programme [6]. Such reviews optimally include representation from all stakeholder groups, including physicians, nurses, laboratory staff and information system support staff, sufficient time for creating locally appropriate policies and procedures and adequate testing and training. Pilot implementation at a hospital may be a useful way to uncover issues for correction prior to data entry into a national haemovigilance system.

Scope, Breadth and Analysis of Reporting

While all haemovigilance systems require a system to capture events directly from the site of transfusion or blood donation, the scope, breadth, analysis of the haemovigilance data and the responsible reporting entity (or entities) may vary (Table 21.2).

Scope

Variations in scope have advantages and disadvantages. For example, while the UK Serious Hazards of Transfusion (SHOT) system limits its scope to only serious events having the largest potential impact on a transfusion recipient [7], such a design may miss events of lesser morbidity that affect a large number of patients or that may be harbingers of more serious outcomes. Systems that capture all transfusion reactions risk developing reporting fatigue, and also might result in numerous, less severe reports obscuring major clinically significant adverse events. The type of event reported/collected varies as well, with some haemovigilance systems receiving only transfusion adverse reaction reports versus systems that receive reports of incidents (i.e. mistakes, errors, deviations or occurrences). As these incidents are part of the transfusion process, they may be linked in some cases with events that have caused harm; a study of transfusion-associated circulatory overload (TACO) found that one in five TACO reactions was due to human error [8].

Including incidents not directly associated with a reaction or untoward outcome may help detect and prevent system problems from harming patients. Failures to adhere to standard procedures may represent human limitations, inadequate training, unique features of a patient's situation or a combination of factors that align with weak points in the transfusion or collection process.

Most notable among incidents reported to haemovigilance systems have been patient and sample identification errors in pretransfusion

Table 21.2 Variations among haemovigilance systems.

Scope	Serious events only All events regardless of severity, including 'near-miss' events Only events causing harm Events related to transfusion recipient safety Events related to blood donor safety Events related to both blood donor and transfusion recipient safety
Breadth	Labile transfusable components Plasma derivative products Cells, tissues and/or organs for transplantation (biovigilance)
Analysis	Local institutional (i.e. hospital or blood centre) level Regional or healthcare system level, allowing benchmarking among all hospitals/blood centres within a category National level International level Benchmarking/comparison with (anonymous) peer institution subset Analysis of incidents and events, with or without access to full details of incidents and events
Reporter into the system	Identified and trained individual who oversees reporting of events into system (France, UK, Quebec) Use of pre-existing hospital transfusion reaction monitoring system that reports to hospital transfusion service and committee (USA) Electronic reporting, with clinician review, from local medical record system

testing. Including 'near-miss' events where the error is detected and remedied and/or where it does not cause harm to the recipient quickly causes such occurrences to become the most commonly reported events in a haemovigilance system. Some haemovigilance systems do not examine product- or process-related incidents unless there is actual harm to the recipient or donor [9]. Although these occurrences may individually appear to be of minor importance, they represent a critical view into the workings of a transfusion system and allow preventive actions to be taken to bolster system safeguards [10].

Including donor operations is integral to a haemovigilance system, because a system that spans from donor vein to patient vein is best positioned to identify systemic problems and inform policy changes. Comparisons of postdonation reactions upon collection of different blood volumes have been possible through donor vigilance, as well as analyses of the rate of postdonation death among donors assumed to be healthy [11].

Analysis

Reviews of transfusion adverse events (TAEs), incidents, and/or transfusion outcomes may occur at the local, national and/or international levels. At the local level (usually within the hospital or blood collection centre), adverse transfusion reactions and donor reactions and rates should be analysed and compared to a larger whole, such as a regional, national or international system, thereby locally benefiting through comparison of its experience to that of others working within the system (e.g. other paediatric hospitals, other mobile blood collection sites). Haemovigilance applied locally can address important local operational and research questions using the experience of a larger number of tracked events. Even a healthcare subunit, such as anaesthesiology or cardiology, may choose to apply haemovigilance definitions to its own practice(s) to better characterise adverse events and their frequency [12,13].

At the national level, a haemovigilance system's large purview can be applied for postmarketing surveillance to assess the safety of

new products and new interventions, such as coronavirus convalescent plasma [14] or pathogen inactivation [15,16].

In addition to analysis of the data from submitted reports, the haemovigilance system itself may be analysed and modified at the national level as needed to enhance its operability. Thus, a haemovigilance system may evolve to enhance its ease of use, increase timeliness of reporting, and reinforce or update data definitions [17].

Standardisation of Data Elements for Data Capture

Haemovigilance systems receive event reports consisting of defined data elements describing transfusion and collection adverse events or near misses, and the associated clinical findings and patient characteristics in blood collection and transfusion therapy. Using established, standardised terminology and definitions for these data ensures comparability of recorded observations and is necessary for meaningful comparisons in data analysis (Table 21.3) [18].

The International Hemovigilance Network (IHN) recognised early on the importance of data standardisation for comparison between systems [19]. Clear, precise definitions resistant to misinterpretation may decrease the chance of misapplying a classification and degrading submitted data. Even with clear definitions of adverse events, misapplication of definitions may occur. Most systems impose a review of reported events to ensure that they meet the reporting system definitions. Such rigour adds robustness to reporting; however, larger systems or those with fewer resources depend on participants' adherence to definitions and accurate data entry. Highly resourced systems may develop computer algorithms to detect, validate and report adverse events, thereby accepting only reports of events that meet requirements for evidence and completeness. Some haemovigilance systems adjudicate reports prior to analysis; for those that lack adjudication, process validation of the application of definitions may be critical at local and national levels [20] and may also occur internationally [21].

Table 21.3 Benefits of standardised, uniform data and terminology.

Permit use for data capture and reporting
Ensure data integrity for comparative analyses
Enable consistent tracking of internal performance over time
Permit benchmarking across institutions
Allow for assessment of outcomes of process improvements

Often incorporated into TAE definitions are criteria that define the reaction, score its severity and determine the imputability or likelihood that the observed event is attributable to transfusion. The International Society of Blood Transfusion (ISBT) Haemovigilance Working Party developed transfusion reaction definitions that may be used to harmonise, achieve commonality and facilitate meaningful comparisons of data between countries. These definitions have evolved and changed as further information and knowledge are gained [17,18,21]. An example of an ISBT transfusion reaction definition is given in Table 21.4.

Data Management

Ease of TAE detection, review and reporting is an essential component of systems with high participation rates. In most circumstances, the detection of a transfusion reaction is dependent on observation and reporting by trained medical personnel. Under-recognition of transfusion reactions is known to occur.

Many haemovigilance systems are converting, or have converted, to allow electronic submissions. An intelligent, web-based data-capture system could display only items pertinent to the case report as it unfolded. Such a context-sensitive system could check for completeness or internal congruity in a case and prevent entry errors. As electronic medical records become commonplace in healthcare, computer processes

Table 21.4 International Society for Blood Transfusion haemovigilance definition, severity score and imputability grade examples [18].

Definition: hypotensive transfusion reaction

- This reaction is characterised by hypotension, defined as a drop in systolic blood pressure of ≥ 30 mmHg occurring during or within one hour of completing transfusion **and** a systolic blood pressure ≤ 80 mmHg.
- Most reactions do occur very rapidly after the start of the transfusion (within minutes). This reaction responds rapidly to cessation of transfusion and supportive treatment. This type of reaction appears to occur more frequently in patients on angiotensin-converting enzyme (ACE) inhibitors.
- Hypotension is usually the sole manifestation, but facial flushing and gastrointestinal symptoms may occur.
- All other categories of adverse reactions presenting with hypotension, especially allergic reactions, must have been excluded. The underlying condition of the patient must also have been excluded as a possible explanation for the hypotension.

Severity

Grade 1 (non-severe): the recipient may have required medical intervention (e.g. symptomatic treatment), but lack of such would not result in permanent damage or impairment of a body function.
Grade 2 (severe): the recipient required inpatient hospitalisation or prolongation of hospitalisation directly attributable to the event resulting in persistent or significant disability or incapacity; or the adverse event necessitated medical or surgical intervention to preclude permanent damage or impairment of a body function.
Grade 3 (life-threatening): the recipient required major intervention following the transfusion (vasopressors, intubation, transfer to intensive care) to prevent death.
Grade 4 (death): the recipient died following an adverse transfusion reaction.

- *Grade 4 should be used only if death is possibly, probably or definitely related to transfusion. If the patient died of another cause, the severity of the reaction should be graded as 1, 2 or 3.*

Imputability

This is, once the investigation of the transfusion adverse event (TAE) is completed, the assessment of the strength of relation to the transfusion of the TAE.
Definite (certain): when there is conclusive evidence beyond reasonable doubt that the adverse event can be attributed to the transfusion.
Probable (likely): when the evidence is clearly in favour of attributing the adverse event to the transfusion.
Possible: when the evidence is indeterminate for attributing the adverse event to the transfusion or an alternative cause.
Unlikely (doubtful): when the evidence is clearly in favour of attributing the adverse event to causes other than the transfusion.
Excluded: when there is conclusive evidence beyond reasonable doubt that the adverse event can be attributed to causes other than the transfusion.

- *Only possible, probable and definite cases should be used for international comparisons.*

and algorithms that comb the records and clinicians' notes of patients receiving blood products may identify clinical determinates of transfusion reactions and flag them for further review, potentially improving reaction recognition and reporting to the transfusion service. The quality of analysis possible from computerised databases depends upon the consistency of the data element definitions established in the database, appropriate application of definitions and accurate data entry.

Most haemovigilance systems are not interfaced with a facility's internal error management software, but centrally coordinated healthcare systems may be able to integrate laboratory information, patient medical records, error management and haemovigilance systems. ISBT128 uniform terminology [22] provides for TAE reactions the traceability that is key for haemovigilance structures. Ideally, data integration of multiple information system sources (hospital, laboratory, blood bank) reduces complexities and duplication [23]. In many cases, this integration and consistency come through regulation of the electronic healthcare software systems [24], which is a target for future development in many countries where neither the

technology nor the regulatory infrastructure exists.

The more detail of an event is captured, the greater the impact on policy development that reports can offer. Capturing detail about where an incident occurred may help identify high-risk clinical areas, and knowing which steps in the transfusion process were vulnerable to error will similarly help direct attention to the part of the transfusion process where improvements may have the greatest impact. Capturing the results of root cause analyses and estimating recurrence probability and impact can help focus attention on critical targets.

For meaningful haemovigilance analyses and comparisons, a denominator is required to turn reported occurrences into rates. This may be the number of units of each component type transfused, the number of whole blood units collected, or the number of pretransfusion specimens tested, depending on the type of data being compared (adverse transfusion reactions, blood donor events or incidents). Ideally, systems are allowed to mature with a few years of well-established reporting rates, before quality assurance projects and interventions are pursued; however, comparisons can be made sooner provided that data structures are reliable and harmonised with the comparators, and limitations of comparisons are clearly stated. Nevertheless, comparisons of event rates may reveal meaningful lessons across institutions and/or countries of different sizes and transfusion activity levels.

Haemovigilance System Limitations

Detection of untoward outcomes that occur long after a transfusion will always be an inherent challenge for haemovigilance systems because of the potential lack of a clear temporal relationship (Table 21.5). For example, identifying transfusion-transmitted infections requires confirming infection in the patient post transfusion, the lack of infection pre transfusion, the same specific pathogen in the blood donor, the incubation period of the pathogen, and the likelihood of acquiring the infection through other modalities. Nevertheless, haemovigilance and biovigilance investigations led to the detection of transfusion-transmitted West Nile virus (WNV) relatively early in the US outbreak; linking poor patient outcome and transfusion exposure of an organ donor would have been more difficult if the incubation period had been longer. An additional utility of a haemovigilance system is real-time assessment of regional risk through donor testing and the subsequent revision of donor screening and testing protocols in response to new information. Haemovigilance systems are not constructed to identify emerging infectious agents or other agents that remain unknown, but can be invoked once risks have been identified.

Table 21.5 Limitations of haemovigilance systems.

Incomplete reporting
Detection of transfusion relationship of late events, including infections
Limited details
Variation in terminology and definitions
Influence of healthcare system's or institution's 'culture' regarding compliance, process improvement and reporting
Use of non-standard definitions and terminology
Inability to track cases back to source documents to ensure that correct and complete reporting has occurred

Lessons Learned from Haemovigilance

As haemovigilance systems have matured, they have provided observational reports with insights into the problems faced by different healthcare systems all over the world and into the risks borne by their donors and recipients.

Common themes from haemovigilance programmes have led to recommendations that have improved transfusion safety. Transfusion of the incorrect unit or component is the most potentially serious system problem, which

may manifest as the patient not receiving precisely the component (sub)type ordered or as a fatal haemolytic transfusion reaction [25]. Inadequate and/or inaccurate patient or sample identification at the time of pretransfusion sampling and at transfusion are frequent problems that defy simple solutions and merit more attention and capable technology [9,10]. Recognition by SHOT of the magnitude of the problem posed by transfusion-related acute lung injury (TRALI) and the high frequency of TRALI cases associated with the plasma of female donors prompted the UK's National Blood Service to reduce that proportion of plasma for transfusion, resulting in a marked reduction in the number of deaths attributable to TRALI [26].

Identification in a single reporting period that TACO was the most common cause of posttransfusion mortality in Quebec [2] prompted increased clinician education about this condition, and additional clinical attention to the complication has led to a decline in its frequency. Data from haemovigilance systems have helped define the frequency of bacterial contamination of platelets, investigate clusters of such incidents and document the effects of patient safety-oriented interventions [27].

Several applications of haemovigilance data from the UK SHOT system have been particularly noteworthy, improving transfusion safety in the UK system and showing the power of haemovigilance when data are applied thoughtfully through evidence-based recommendations (see Further Reading).

Reports of mistransfusion due to patient/sample misidentification continued unabated until the recognition that descriptions of the problem alone would not effect an improvement. Evolution of the SHOT recommendations included near-miss events, not just mistransfusions that cause patient harm. Subsequent implementation of augmented approaches to patient, sample and unit identification was associated with a decline in the number of reported deaths due to mistransfusions. Similar results of interventions have been reported from other systems, such as in France, where ABO-incompatible transfusions were reduced by three-quarters [28]. SHOT continues to refine its recommendations on this topic to address patient safety concerns [7], demonstrating that improvement to patient safety is an iterative process.

Call to Action and Future Directions

While many nations have adopted elements of haemovigilance, not all countries have haemovigilance systems. With the framework and lessons learned from haemovigilance programmes, newly formed haemovigilance systems may both learn from and contribute to the growing body of knowledge and data on unexpected and adverse events associated with blood donation and transfusion. Transparency and sharing of data analysis are key to the growth of the field and furthering advances in blood collection and transfusion safety. The WHO Aide-Mémoire, 'National Haemovigilance System', encourages all countries to develop and implement a national haemovigilance system to improve blood safety worldwide [29].

As new technologies emerge through tablets and smartphones, opportunities for new reporting tools may be added to the haemovigilance armamentarium. Computerising formerly paper-based processes reduces inefficiencies of manual data entry, whether at the level of the hospital/blood centre or in the haemovigilance system office. Enhancements to current healthcare electronic information systems such as electronic medical records and/or integrated blood bank/laboratory information systems could provide a means for such direct data entry into a haemovigilance system and thereby ease the reporting burden. Clinical decision support tools that have been designed for patient blood management could be repurposed for haemovigilance and offer guidance and direction for providers in identifying adverse events in transfusion therapy in 'real time'.

Haemovigilance programmes bring both evidence-based and intangible benefits to patients, donors and providers. They will continue to evolve in the design of data-reporting mechanisms, structure and definitions of data, and offer data-driven improvements in transfusion safety.

KEY POINTS

1) Defining and measuring transfusion adverse events combined with data analysis and concerted actions improve transfusion systems and reduce transfusion risks.
2) Haemovigilance systems are most effective when based on data reported using standardised nomenclature and definitions.
3) Including 'near-miss' incidents in haemovigilance reporting provides insights into weak points of the transfusion process and opportunities to improve the system by reducing the potential for error.
4) The most dangerous problem reported across multiple haemovigilance systems is the transfusion of an incorrect blood component, often relating to sample and patient identification errors.
5) Identifying serious problems followed by action(s) directed at their cause can improve transfusion safety, as seen in the steps taken to reduce the frequency of TRALI- and ABO-related acute haemolytic events.
6) Haemovigilance systems have led to determination of causality of transfusion adverse events and identification of interventions to reduce transfusion risk.

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22 Donors and Blood Collection

Marc Germain[1] *and Pierre Tiberghien*[2]

[1] *Héma-Québec, Quebec City, Quebec, Canada*
[2] *Établissement Français du Sang, La Plaine Saint-Denis; Inserm UMR 1098 RIGHT, Université de Franche-Comté, Établissement Français du Sang, Besançon, France*

Collecting blood from people for transfusion to others is an essential part of healthcare. A developed healthcare system needs to provide approximately 27–40 therapeutic units of red cells and around six therapeutic doses of platelets and of plasma annually per thousand of the population it serves. Whole blood donation as well as apheresis will also provide plasma for the manufacturing of medicinal products such as immunoglobulin, albumin and coagulation factors.

Blood Donors: Altruistic, Directed, Payback and Paid

People can be motivated to donate blood (including plasma intended for the preparation of medicinal products) in three different ways:

- As an altruistic act.
- As a direct response to the needs of an individual they care about.
- For an economically valued reward.

All three methods are in wide use today, and all have their drawbacks. However, societies that succeed in establishing a mature programme of altruistic donations generally gain a more secure and stable supply of safer blood for transfusion. Considering the ethical issues regarding blood donation, the World Health Organization (WHO) as well as the Council of Europe and the International Society of Blood Transfusion (ISBT) call for voluntary non-remunerated donations. There is furthermore compelling evidence that the incidence and prevalence of infectious diseases are higher among donors who donate for personal economic gain. Also, individuals who are directly approached by a relative or friend to donate for a particular patient are more likely to withhold critical information about their personal infectious risk history.

Motivation, recruitment and retention of altruistic donors are not easy or cheap. In most developed nations, 5% or less of the population donates per year. Establishing and maintaining a mature altruism-based blood donation and collection programme require a high degree of social cohesion and an immense effort in education and communication. Many successful national or regional programmes based on altruism were set up around the middle of the twentieth century, at a time of national need in conflict or post conflict. Countries that did not establish altruism-based blood services to begin with have tended to find it much more difficult to establish one afterwards. Huge efforts are currently being made to redress this

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throughout the developing world (see Chapter 27).

Paying blood donors will provide a supply of blood, but it requires enough people in the population for whom the payment on offer provides sufficient motivation. In more developed economies, the balance of high demand for blood for transfusion with limited numbers of people who will be motivated by the rewards on offer often makes paying for donations an inadequate strategy. In addition, paying for blood also undermines the alternative, more successful motivation of altruism in these economies.

As mentioned earlier, paid donors are, in general, a less safe source than volunteer donors. In an analysis of 28 published data sets, it was found that while the incidence of disease markers had diminished over the years between 1977 and 1996 for paid and unpaid donors alike, unpaid donors were on average 5–10 times safer than paid donors and that this difference had not changed over time [1]. It is assumed that people who have no great incentive to donate other than genuine regard for their fellow humans will tend not to withhold risk information. People who need money or items of small economic value at the level they may be offered by blood services are more likely to withhold relevant risk information. In addition, increased at-risk exposure from drug addiction or sex working occurs more frequently at the lower economic margins of a Westernised society.

A system of directed and payback donations, where donors are recruited among the relatives and friends of the patient requiring blood transfusions, also provides some supply. Such an approach will generally be insufficient to support a well-developed healthcare system and is prone to incite covert payments to donors, including professional donors. However, some experts believe that a system relying on donations by family members and acquaintances may offer a viable and safe alternative in developing countries where a fully altruistic model is not yet in place.

European Union (EU) Directive 2002/98/EC (see Further Reading) instructs member states to promote community self-sufficiency in human blood and blood components and to encourage voluntary unpaid donations of blood and blood components. However, it is worth noting that the vast majority of the world supply in plasma intended for fractionation still comes from remunerated donations, mainly from the USA but also from other countries in Europe and elsewhere. In this context, not-for-profit blood establishments in Europe and other regions are currently increasing plasma collection intended for fractionation in a voluntary, non-remunerated donation setting. By favouring less frequent donation by a larger donor base, this approach allows for the collection of plasma with higher protein content, increases donor protection by reducing donation burden, while providing a more robust donor base in case of crisis.

Even in the altruistic model, the underlying motivations to donate blood are likely to be multifaceted and complex. Fortunately, blood donation behaviour is increasingly being studied by psychologists, sociologists and behavioural economists. These insights will help blood collection agencies to attract and retain donors, beyond the well-accepted strategies that successful programmes already apply: active communication with the donor from the beginning, making donation convenient, reducing donor anxiety and adverse reactions, having well-trained and motivated staff and encouraging temporarily deferred donors to return as soon as possible following the expiry of their deferral period [2].

Challenges remain in recruiting donors from ethnic minorities. Populations of different ancestries have different disease patterns with different transfusion needs, notably in relation to different red blood cell antigen frequencies. Added to this, data suggest that migrants tend not to become blood donors in their new country. A number of factors may contribute to the proportionally low representation of minorities in the donating population, including culture,

lack of social/ethnic identification, fear and lack of information.

Risks to the Blood Donor

Blood donation, be it a whole blood collection or an apheresis procedure, is generally very safe. Most people can readily tolerate venesections of approximately 10% of their blood volumes without apparent harm or significant physiological compromise. However, it is not a trivial undertaking and it requires considerable care to minimise the risk to the donor, who can almost never expect any health benefit from the donation. The risks associated with blood donation are listed in Table 22.1.

Table 22.1 Main adverse events or reactions in blood donors.

	Frequency (per 100 000 donations)*		
	Minor to life-threatening adverse events	**After exclusion of minor adverse events**	**Prevention**
	1000–3000	**100–200**	
Fainting (vasovagal) reactions	Fainting reactions are more frequent in young donors and female donors; delayed (off-site) fainting is under-reported. Active surveillance suggests a fainting reactions frequency of ≈ 5%		Adapting collection volume to body mass index Active surveillance during and immediately after donation, muscle tensing (to prevent on-site fainting reactions mainly), hydration and salt intake (to prevent off-site fainting reactions mainly)
Haematoma	100–300	10–20	n.a.
Arterial puncture	4–5	4–5	n.a.
Nerve injury (direct or haematoma-related)	10–20	2–3	n.a.
Local pain	18–20	1–2	n.a.
Thrombophlebitis	0.3–0.5	0.3–0.5	n.a.
Citrate toxicity (apheresis only)	500–800	10–30	Calcium (per os or IV) administration upon hypocalcaemia-related symptoms

Iron deficiency†	Under-reported
	• Adult donors:
	  ○ First time/reactivated: 24.6% in females and 2.5% in males
	  ○ Repeat donors: 66% in females and 48.7% in males
	• Age 16–18 donors:
	  ○ First time: 52.3% in females and 9.2% in males
	  ○ Repeat: 69.9% in females and 30.6% in males
			Donation deferral if low Hb and/or low ferritin; iron supplementation; increased intervals between blood donations

(*Continued*)

Table 22.1 (Continued)

	Frequency (per 100 000 donations)*		
	Minor to life-threatening adverse events	**After exclusion of minor adverse events**	**Prevention**
	1000–3000	**100–200**	
Iron deficiency anaemia[†]	Under-reported Estimate: approximately 18% of donations with post-donation laboratory values consistent with iron-deficient anaemia; 3 : 1 female-to-male ratio		
Protein depletion (plasma apheresis)	Under-reportedHigh-frequency (≥ 1/week) plasma apheresis: up to 27% of donors with a plasma IgG level below a threshold of 5.8 g/L[‡]		Low-frequency plasma apheresis Regular plasma protein and IgG level measurements, donor deferral if plasma protein or IgG level falls below a threshold (e.g. IgG < 6 g/L in the 20th edn Blood guide, EDQM, Council of Europe, https://www.edqm.eu/en/blood-guide)

*Adapted from French (ANSM 2018), American (AABB 2018) and Australian (Red Cross 2015–16) haemovigilance reports.
[†]Adapted from the AABB donor iron deficiency risk-based decision-making assessment report (2018).
[‡]Sipla studies (Schulzki et al. 2006; Kiessig et al. 2013); see Further Reading.
AABB, Association for the Advancement of Blood and Biotherapies; ANSM, Agence nationale de sécurité du médicament et des produits de santé; EDQM, European Directorate for the Quality of Medicines & HealthCare; Hb, haemoglobin; Ig, immunoglobulin; IV, intravenous; n.a., not applicable.

An internationally accepted description and classification of adverse events and reactions was proposed by the International Haemovigilance Network (IHN) and the ISBT in 2004 and has been refined since then, with the more recent contribution of the Association for the Advancement of Blood and Biotherapies (AABB) to this initiative (see Further Reading). It classifies complications into two main categories: those with predominantly local symptoms and those with predominantly generalised symptoms. Complications specific to apheresis procedures are categorised separately. Complications are further graded into mild, moderate and severe and assigned an imputability score for the likelihood of blood donation being the cause of the reaction.

Some complications are specific to apheresis donations, including citrate reactions, haemolysis, air emboli and allergic reactions to ethylene oxide used in the sterilisation of the harness. Most blood establishments will only report citrate effects if they are severe or if they result in the donation being discontinued, the mild reactions being very frequent (metallic taste and tingling in the lips).

Longer-term consequences of donation, such as iron depletion with or without associated anaemia, are usually not tallied in routine donor hemovigilance reports.

The overall incidence of complications directly related to blood donation is often quoted as being approximately 1%, though the true reaction rate may be higher. One study, where information on adverse events was actively sought on follow-up rather than relying on passive collection of spontaneous reports by donors, reported that from 1000 randomly selected donors, three weeks after donation 36% had had one or more adverse

events [3]. Of complications collated by the IHN/ISBT Working Group, 99% belonged to four categories: vasovagal reactions (86% of all complications), haematomas (13%), nerve injuries (1%) and arterial punctures (0.4%) [4].

Rarely, severe complications arise, such as accidents related to vasovagal reactions and nerve injuries with long-lasting symptoms. Vasovagal reactions that occur after the donor has left the session, estimated to represent 10% of all these reactions (with some significant under-reporting), are of particular concern and may have on rare occasions led to accidental deaths. A retrospective analysis of Danish data relating to 2.5 million donations found that severe complications occurred with an incidence of 19 per 100 000 procedures [5].

Young age, first-time donor status and low total blood volume are independent predictors of higher reaction rates. Complication rates of 10.7% in 16- and 17-year-olds, 8.3% in 18- and 19-year-olds and 2.8% in donors aged 20 years and older have been observed. Syncope occurred in 4 in 1000 donations and injury in 6 in 10 000 donations in 16- to 17-year-olds and almost half of the injuries that occurred in American Red Cross regions involved whole blood donors in this age group [6].

It is unlikely that the risks to blood donors can ever be reduced to zero, which places a significant ethical burden on blood services to use their best endeavours to reduce the risks. This includes the careful collation and analysis of data on the incidence and nature of adverse events or reactions, and the sharing and comparison of these data among blood services, with the goal of identifying and promoting best practices. The uneven risk-to-benefit ratio for blood donors also places an ethical responsibility on healthcare givers to avoid wastage and unnecessary use of blood transfusions.

Several strategies can be used to reduce the risk of complications occurring during and after donation, both to ensure the health and well-being of blood donors and also to sustain an adequate blood supply. Even minor reactions discourage donors from donating again and more severe reactions profoundly decrease the return rate of donors. Best practices include good needle insertion techniques; predonation education; optimising the session environment; appropriate selection criteria (particularly as regards estimated blood volume); vigilant supervision of donors; hydration before and during donation, including isotonic beverages; distraction techniques and muscle tension during phlebotomy; and postreaction instructions to donors [7]. In addition to donor deferral for low haemoglobin, iron depletion and resulting anaemia may be addressed by increased donation interval as well as iron supplementation, ideally guided by ferritin determination.

Plateletpheresis-associated lymphopenia has been reported in frequent platelet donors. Such lymphopenia involves both CD4 and CD8 T cells and may be long-lasting. The mechanism most likely involves leucocyte retention during the apheresis procedure. Early assessment suggested that this lymphopenia is probably not harmful. However, a recent study relying on the analysis of large administrative health databases suggests that donors undergoing plateletpheresis using a leucocyte-reduction system chamber may be at a (modest) increased risk of immunosuppression-related infections and common bacterial infections in a dose-dependent manner [8]. Although these last findings rely on a small number of events and therefore require confirmation, they highlight the importance of diligently approaching donor protection, without any preconceived notion about the impact of donation procedures on donor health. They also illustrate the potential for data linkage between donor and general population health databases to explore potential long-term adverse events in blood donors. Another situation in which this approach might be useful is to assess the potential health impacts of chronic plasma protein depletion in high-frequency plasma donors.

Donor Selection and Deferral

Prospective blood donors are subjected to a process, often specified in national legislation, intended to minimise the risks to the donor and to the eventual recipient of the donated blood. This involves a donor history, including any recognisable risk in the donor, for transmitting infectious agents to the recipient. Infectious risks from donors are listed in Table 22.2, along with available mitigation strategies.

In some services the donor undergoes some cursory form of physical examination, but the value of this examination is doubtful, at least among altruistic donors.

Donors generally undergo a measurement of their haemoglobin level, either prior to the donation or, in some countries, on a sample taken at the same time as the donation. This is either from a skin puncture ('capillary sample') or a venous sample. This measurement of haemoglobin serves two purposes: it provides some protection to the donor against having a pre-existing anaemia made worse by donating; and it helps ensure that the final therapeutic product will have a minimum red cell content. The cut-off levels for the allowable haemoglobin level in the donor vary between blood services and regulatory authorities and are empirically derived. Often, as in the EU rules (Directive 2004/33/EC; see Further Reading), a different level is used for males and females, with the allowable minimum haemoglobin level set higher for males. Haemoglobin levels vary in the same individual between capillary and venous blood [9], with the seasons and the time of day and with posture and activity. In addition, this measurement does not provide protection against non-anaemic iron deficiency. An updated systematic review of the literature pertaining to donor deferral due to low haemoglobin level has recently been published [10].

In some jurisdictions, donor deferrals may be specified by law. In the EU, the specifications are generally interpreted as a minimum requirement by the member states or the national blood services (see Further Reading for a complete list of those deferrals). In the USA and other jurisdictions, in contrast, the specifications are generally regarded as a maximum requirement by the blood service operators. For many specific clinical conditions, the requirements may vary between jurisdictions, mainly because of the lack of scientific data to support or negate the value of a given deferral criterion. Very often, such criteria are based on clinical judgement, with a variable degree of precaution. For example, the risk of donating blood for people with diabetes remains controversial, although most jurisdictions accept donors with well-controlled type 2 diabetes. When it comes to protecting donors, the general tendency is to exclude from donation those with serious, chronic medical conditions. The same principle applies to protecting recipients; for example, some jurisdictions exclude donors with a history of malignancy, although others will accept them after long-term remission, based on accumulating evidence that transfusion has never been shown to transmit this condition.

There is a long list of infectious diseases that may pose a risk of transmission by transfusion and for which deferral criteria should be applied, either on a temporary basis (e.g. West Nile virus, Q fever, tuberculosis, malaria) or permanently (e.g. human immunodeficiency virus [HIV], hepatitis B virus [HBV], babesiosis). For some infections, the epidemiological context may justify the application of more stringent criteria, for example when there is a localised outbreak, as happened in certain regions of the world with the Chikungunya virus. Of note is that, although the virus is highly prevalent in several jurisdictions, no case of transfusion-transmitted Chikungunya has been reported up to now. Differences between mosquito-mediated intradermal infection and intravenous infection for this disease as well as for other arbovirus diseases may contribute to a low transfusion-associated risk. Also, a deferral may not be required for certain infectious risks when the donation is only for

Table 22.2 Infection risks from blood donors.

Categories of risks	Examples of infections	Donor deferrals that may reduce risk
Window-period infections: a donor is infectious with an agent for which the donation is routinely tested, but the infection was acquired so recently that the donor does not yet have detectable infectivity in the blood	HIV-1 and -2, HCV, hepatitis B	Excluding at-risk donors identified by questions about risk activities in the recent past, e.g. recent at-risk sexual activity, recent tattoos or piercings or recent invasive procedures
Infections for which donors are not routinely tested	Malaria, West Nile virus, Chagas' disease, visceral leishmaniasis, dengue, Zika	Deferring donors, where possible, on the basis of travel or previous residence. This is very difficult in areas of high prevalence and endemicity, and requires additional testing where possible
	Any recently acquired infection that the donor has not yet cleared and that may have a viraemic or bacteraemic phase	Deferring donors on the basis of a recent history of any febrile illness; deferring donors who have recently had a live virus vaccination
Risks from unrecognised, yet-to-emerge infectious agents	In the past HIV and HCV were extensively spread by blood transfusions before the true nature of the diseases became apparent. A similar fate could have arisen with vCJD	Deferring donors with behaviours strongly associated in the past with the early and extensive spread of emerging diseases with long incubation periods, such as sex workers, intravenous drug users and men who have previously had sex with men. However, this approach is highly debatable, given that many of the recently emerging pathogens (e.g. hepatitis E virus, West Nile virus, Zika, vCJD) are not associated with these behaviours
		Deferring donors who have previously received blood transfusions. This is also controversial, for the same reasons as given above. Several jurisdictions do not apply a permanent deferral to these donors
Risk from transmissible spongiform encephalopathies	All prion diseases are considered to have the possibility of an infectious blood phase	Deferring donors who have a strong family history of spongiform encephalopathy; excluding donors who have been treated with human-derived pituitary hormones or dura mater
		Outside the UK and Europe, deferral on the basis of residence in higher-risk countries during the bovine spongiform encephalopathy epidemic
		In some European countries, previous recipients of blood transfusions are excluded to try to limit the risk of transfusion-transmitted vCJD or other diseases that are currently undetected

HCV, hepatitis C virus; HIV, human immunodeficiency virus; vCJD, variant Creutzfeldt–Jakob disease.

plasma intended for fractionation, because of the inherent inactivation capabilities of the fractionation process.

Deferral rates vary in different blood services, ranging in the EU from 0.5% to 25.2% of donors, with a mean of 10.9%. The lowest deferral rates are in countries where the public knowledge of blood donation selection criteria is high – where donors may register online and complete an eligibility questionnaire in advance. A low haemoglobin level is typically the most frequent reason for deferral, accounting for nearly 40% of deferrals.

Iron Deficiency in Blood Donors

Blood donation results in a significant iron loss of approximately 200–250 mg per donation. Both iron deficiency causing anaemia and iron deficiency in the absence of anaemia are common among donors, particularly in females of childbearing age. Iron depletion below a ferritin level of 12 µg/L can be present even when there is no evidence of iron-deficient erythropoiesis. It may cause poor concentration and sleep disturbances, and has been associated with restless legs syndrome. Iron deficiency can also arise in donors of plasma or platelets by apheresis due to the red cell losses that result from the procedure. A study of Australian blood donors showed that 5.3% of males and 18.9% of females who met the EU criteria for haemoglobin levels were iron deficient, as defined by a serum ferritin level of less than 12 µg/L. Iron deficiency among the general female population in Australia is 5–7% and is negligible among the general male population [11]. Similar findings were noted in a US study [12].

In blood donors, who are considered healthier compared to the general population, the true clinical significance of iron deficiency without anaemia has not been studied extensively and remains largely undetermined. One study has found that female blood donors with lowered iron stores, when compared with donors with normal iron stores, are more likely to report pica, the craving and compulsive eating of non-food substances [13]. However, no association has consistently been found between low iron stores and other potential complications of iron deficiency, including restless legs syndrome and decreased cognitive function. A Danish study conducted among 16 375 blood donors did not show any association between self-reported health-related quality of life and iron status [14]. A large, randomised trial in the UK recently showed that more frequent donation reduced iron stores and increased deferrals for low haemoglobin; however, there were no differences in quality of life, physical activity or cognitive function [15].

Iron deficiency among donors may be prevented or treated by adequate intake of oral iron. However, optimum regimens for blood donors have not been generally defined and practice varies considerably. Options include regular measurement of blood or plasma indices of iron deficiency, routine provision of iron supplements and dietary advice. Due to the risk of serious iron toxicity in children who accidentally take a donor's iron tablets, iron should be dispensed with adequate precautions.

Men Having Sex with Men and Blood Donation

The deferral rules applied to men who have sex with men (MSM) have long been debated and criticised by those who view such policies as unduly discriminatory. The EU Directive does not include any specific requirement in this regard, except to mention that persons with high-risk sexual behaviours should be excluded from blood donation. For a long time, most countries have applied a permanent deferral to MSM, who, as a group, remain at much higher risk of HIV compared to the general population. However, several countries have changed from a permanent to a temporary deferral after the last male-to-male sexual contact, without any negative impact on product

safety. Some countries even allow donations from sexually active MSM, with some restrictions, in particular with respect to the number of sex partners; other countries are actively contemplating similar policies to be implemented in the near future. Given the high profile of this issue in many jurisdictions, it is likely that deferral rules for MSM will continue to evolve or at least remain the object of heated discussions.

Blood Collection/Donation Process

The entire donation procedure needs to be controlled within a functioning quality system, while maintaining the humanity of the process, and especially the dignity of the donor. The venue must be clean and warm, but not excessively so, uncluttered, bright and without excessive noise. Staff should not be distracted or distressed by extraneous events. There must be appropriate space available for confidential discussions between donors and staff. The flow of the donor from reception through registration, interview, haemoglobin check (if done) and venesection should be orderly and unidirectional.

Several blood services do not take a blood collection from a donor on their first attendance. Instead, they take a sample for blood group, blood count and virus screen. This practice provides some protection against window-period donations from people who are donating for the purposes of getting an HIV or hepatitis test. It is, however, very costly, especially when a significant proportion of blood comes from first-time and once-only donors.

Donors may be recruited or retained to donate for apheresis as well as, or instead of, whole blood. Apheresis may be for red cells, usually as a double dose from larger donors, platelets or plasma, or combinations of these. Donor acceptance or deferral criteria are similar to those for whole blood donors, though plasma (for fractionation) donors may be exempted for some infectious risks. Platelet and plasma donation intervals are shorter. Much of the plasma used in the manufacture of blood components comes from apheresis donors. In the USA, most of them are paid and can donate up to twice weekly. Since the early 1990s, blood component manufacture has had a very good safety record regarding transmission of infectious diseases. This has been achieved by increased donor screening and deferral procedures, advances in testing and effective methods of pathogen inactivation. As things stand at present, the supply of plasma-derived medicinal products worldwide, notably immunoglobulins, could probably not be maintained without paid plasma donation.

Obligations to Donors

Although donors are well and are not seeking care, they are nevertheless subjected to a healthcare intervention. The blood service has an ethical obligation to them from the very start of the first attendance. The service's main duty of care is to the recipient of the donation, but it also has obligations to the donor.

The donor has a right to:

- Confidentiality and autonomy.
- Informed consent.
- Protection from harm – including not being made to feel unhealthy when they are outside donation specifications.
- Receive the results of tests when these are of significance to their health.
- Receive direction and counselling around the results of such tests.
- Be protected as much as possible from adverse events or reactions.

In turn, donors are required to:

- Identify themselves correctly.
- Be truthful in their answers to the screening questions.
- Inform the blood service if any change arises in their health after they have donated.

Donors also have some rights in relation to the use of their donation – the consent that they give must include the possibility that the donation may not be used for the therapeutic use that they assume, but that it might expire unused or be used for control purposes. Where a unit of blood is collected specifically for control, test or calibration purposes, explicit consent should be sought. Lastly, donors have a right to expect that healthcare providers will ensure ethical and appropriate use of this unique medicinal product.

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KEY POINTS

1) Voluntary, non-remunerated donation remains the preferred approach to collect blood; however, plasma intended for fractionation is still largely obtained from paid donors.
2) Iron deficiency is common among donors; it can occur in the absence of anaemia and even of iron-deficient erythropoiesis and might result in adverse health outcomes, although the true extent of this potential problem has yet to be determined.
3) Fainting reactions in blood donors are frequent, most often mild, but on rare occasions may result in very severe consequences. Further efforts to reduce such risks are warranted.
4) High-frequency plasma donation might be associated with a higher risk of poorly assessed long-term adverse events.
5) Assessing the donor is a critical manufacturing step in the preparation of the final therapeutic product.
6) The donor has a right to confidentiality and autonomy, informed consent and protection from harm.

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23 Blood Donation Testing and the Safety of the Blood Supply

Mindy Goldman[1] *and Steven J. Drews*[2]

[1] *Canadian Blood Services; University of Otttawa, Ottawa, Ontario, Canada*
[2] *Microbiology, Canadian Blood Services, Edmonton; Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada*

This chapter describes laboratory testing of blood donations, including:

- Red cell serological testing.
- Microbiological testing and donor follow-up.
- Operational and quality control issues.

Red Cell Serological Testing

Every blood donation is tested for the following:

- ABO blood group.
- Rh D antigen.
- Presence of irregular red cell antibodies.

These tests are necessary for safe transfusion practice to reduce the risk of premature destruction of transfused donor red cells in a recipient's circulation due to immunological incompatibility. Correct ABO blood group typing is critical, since naturally occurring antibodies can cause intravascular haemolysis and severe transfusion reactions if incompatible red cells are transfused. The D antigen is the most immunogenic antigen and D– recipients, in particular D– females of childbearing age, are usually transfused with D– red cells to avoid alloimmunisation. Red cell phenotyping or genotyping may be performed for other antigens, such as Rh C/c and E/e, K, S, s, Fy^a/Fy^b, and Jk^a/Jk^b [1,2]. More extensively typed red cells are needed for transfusion support of patients who have produced alloantibodies or are receiving prophylactically matched units to avoid alloimmunisation (e.g. thalassaemia, sickle cell disorders). In some countries, such as France, the UK and the Netherlands, females of childbearing potential are routinely transfused with K– units. Some blood services, such as the National Health Service Blood and Transplant (NHSBT) supplying hospitals in England and North Wales, perform extended Rh and Kell phenotyping on all donations; others perform phenotyping on selected units. Increasingly, molecular methods are being used to screen donors for multiple blood group systems or for absence of a high-frequency antigen, such as U.

Extended phenotyping or genotyping may be performed on selected groups of donors, such as individuals of Afro-Caribbean origin, to meet the needs of sickle cell anaemia patients for antigen-matched units. Additional phenotyping results may be printed on the red cell component label, to assist hospitals in selecting appropriate red cell units.

Practical Transfusion Medicine, Sixth Edition. Edited by Michael F. Murphy, David J. Roberts, Mark H. Yazer and Nancy M. Dunbar.

ABO and D Grouping, and Detection of Red Cell Antibodies

Tests are carried out on anticoagulated venous blood samples collected at the time of donation, identified by a unique bar-coded identification system, which in most countries is an International Society for Blood Transfusion (ISBT) 128 number.

Donor red cells are tested with monoclonal anti-A and anti-B capable of detecting clinically relevant A and B subgroups (forward grouping). A reverse grouping is performed by testing the donor plasma with A_1 and B reagent cells. D grouping is often performed by testing donor red cells with two different highly sensitive monoclonal anti-D reagents. In many countries, D– first-time donors undergo further testing to confirm that they are D–. Use of sensitive reagents and repeat testing are carried out to optimise the detection of weak or partial D– bearing red cells, including category DVI, so as to correctly label these units as D+. Some of these donors may be considered as D– in a different context, for example testing done in prenatal or pretransfusion testing settings.

Blood services use automated testing systems where samples are divided into separate microtitre plate wells, results are read photometrically and the pattern of results obtained analysed by microprocessors to establish the ABO blood group. The forward and reverse ABO testing results must be concordant to assign a donor blood group, and for repeat donors ABO and D test results must be concordant with historical records.

Donor samples are screened for the presence of red cell antibodies that could cause reduced red cell survival or haemolysis when transfused into antigen-positive recipients. The methods used for screening generally only detect high-titre antibodies. Donor plasma is mixed with group O R1R2 red cells that are positive for clinically significant red cell antigens. In general, only antibodies reacting in the indirect antiglobulin test (IAT) are considered to be clinically significant. Initial screening is performed using an automated testing system; manual testing may then be used to determine antibody specificity. In most cases, donations with non-specific, clinically insignificant or low-titre antibodies may still be released for transfusion, since during component preparation the amount of antibody-containing plasma will be very small and diluted with an additive storage solution.

In some blood services, such as the NHSBT, blood for neonatal transfusion is tested for red cell antibodies to a higher level of sensitivity than standard testing for all other blood products, in order to further minimise the very small risk of transfusion reactions due to passive transfer of antibodies.

High-Titre Anti-A and Anti-B

Some donors have high titres of anti-A and/or anti-B that could cause lysis of A cells and, more rarely, B cells, particularly where large volumes of plasma are transfused [3]. Under routine conditions, recipients should receive group-specific or AB plasma to avoid haemolytic reactions. However, in emergency transfusion situations, there is increasing evidence demonstrating the serological and clinical safety of transfusing low-titre group O whole blood (LTOWB) or group A plasma in a minor-incompatible manner. Most recipients receive group-specific red cells. However, group O red cells may be transfused to neonates and patients with no known blood group requiring urgent transfusion. Because most red cell units are stored in additive solutions, the amount of plasma transfused is small, so risk of haemolysis is very low.

Since platelets have a short shelf-life, it is difficult to provide group-specific transfusions. A small number of cases of haemolysis have been reported, particularly in group A paediatric/neonatal recipients receiving group O platelets.

Some blood services or hospital blood banks screen selected or all donors for high-titre haemolysins. Low-titre products can then be used in clinical situations described above. Plasma can be screened for high-titre

haemolysins by observing the reactions between donor plasma and a diluted sample of reagent A_1B red cells; products that are under a predetermined cut-off level can be labelled accordingly. This can be done manually or using automated systems; however, there is no standard testing method or clear acceptable cut-off titre shown to prevent all haemolytic reactions.

Supplementary Testing

Occasionally anomalies appear in serological testing, making interpretation of results difficult. For example, it has been estimated that 1 in 10 000 blood donors has a positive direct antiglobulin test (DAT), which could interfere with some of the above assays or give a positive result on hospital cross-matching. Donors with positive DAT results on several donations may be deferred.

Testing for HbS may be performed on units used for transfusion support for patients with sickle cell disease or neonates receiving exchange transfusions. Sickle trait (HbAS) blood significantly interferes with the function of some filters currently used for leucocyte reduction and HbAS units do not freeze well using current methods.

Donors with test results that may be of clinical importance should be informed of their results, including donors with repeatedly positive DAT results and donors with HbAS. Donors with rare blood groups or red cell alloantibodies are also usually informed by the blood service.

Microbiological Testing of Blood Donations and Donor Follow-Up

Infectious agents transmissible by transfusion are described elsewhere in this book. Donor selection criteria and deferral of at-risk individuals are important steps in reducing transfusion-transmitted infections. Laboratory testing may not be available or feasible for some agents, and then donor criteria are the only means of deferring at-risk donors. Where testing is available, donor criteria are still important, particularly for recently infected donors, who may be asymptomatic and have negative test results but are infectious. This interval, when testing is negative but transfusion may transmit infection, is known as the 'window period'. Donor selection criteria combined with highly sensitive testing can reduce the residual risk of transfusion-transmitted infections substantially [4–7].

Samples

Infectious disease testing is carried out on serum or plasma venous samples collected at the time of donation and sent to highly automated, centralised donor testing laboratories. Automation approaches included liquid handlers, specimen poolers, nucleic acid extraction and nucleic acid amplification test (NAT) and serology test platforms [8,9]. As with blood grouping, correct sample labelling is critical to ensure result traceability. Serological tests are performed on individual donor samples, but NAT is often performed on pools of samples, with resolution of reactive pools to individual specimens [10]. In resource-poor countries with a high incidence of infectious donors, point-of-care rapid tests may be used on the clinic site [11].

Testing Process and Donor Management

Sensitivity and specificity are important test attributes. Sensitivity refers to the ability of the test to identify truly infected individuals. From the perspective of the transfusion recipient, sensitivity is the most important criterion for a screening test (i.e. the test will accurately identify infected donors). Specificity refers to the ability of the test to identify correctly donors who are not infected. Specificity is important to avoid discarding donations from safe donors and deferring healthy donors. Although most currently used screening assays show remarkably high levels of specificity and sensitivity,

in countries with low prevalence rates of infection it is essential that additional assays are available to confirm infection.

Principles of Investigating a Repeat Reactive Sample

If an initial screening test is reactive, it will be repeated in duplicate. If both repeat tests are negative, the result is considered negative, the donation is used, and the donor may continue donating. If a repeat test is again reactive, the donation is discarded, and confirmatory or supplementary tests are performed to establish whether the screening test result represents a true positive. Confirmatory testing is important for donor counselling, epidemiological tracking and public health reporting. Since donors constitute a low-prevalence population, despite the high specificity of screening tests, many repeatedly reactive screening tests are not confirmed positive on supplementary testing (termed false-positive or non-specific reactions). These donors are deferred; some blood services permit donors to be retested after a defined deferral period and if negative, to be reintegrated as donors.

Blood services must have policies for notifying donors with repeat reactive test results. In some countries, the law requires reporting of an infected person for some infections, such as hepatitis B virus (HBV), to public health authorities. For donors with false-positive test results, it can be difficult to explain that although the test almost certainly represents a false-positive reaction, the individual may be deferred.

When a donor is found to be repeat reactive, components from previous donations that are in inventory will be retrieved and discarded. If the donor is confirmed to be infected, recipients of earlier components from the donor will be identified and offered relevant testing, a process termed 'lookback'. With testing improvements, the likelihood of identifying an infected recipient on lookback investigation is vanishingly small.

Principles of Infectious Disease Testing

Screening tests may detect host immune responses (such as antibody to hepatitis C virus, HCV), a microbial antigen (such as the hepatitis B surface antigen, HBsAg) or microbial nucleic acid (NAT). Test algorithms may involve both serology and NAT for an individual microbe. Testing for bacterial infections is covered elsewhere in this book.

Immunoassays

Immunoassay principles using enzyme or chemiluminescent techniques form the basis for infectious disease testing. Donor plasma is incubated over a solid phase where an antigen–antibody interaction occurs. After incubation and washing, the products of the antigen–antibody interaction are detected by a revealing agent. A conjugate linked to an enzyme such as peroxidase can be detected photometrically after the addition of substrate, which produces colour. Alternatively, the optical device may detect photons emitted by a chemiluminescent reaction. Assays may involve detection of antibody, antigen or both, often referred to as 'combo assays', in a single reaction.

Immunoassays depend on the interaction between microbial antigens and antibodies. Monoclonal antibodies with high avidity directed at 'conserved' microbial antigen epitopes are used. However, mutations in the target epitope may render the antigen undetectable. HBsAg assays must be able to detect known mutants, especially the vaccine escape variant G145R [12].

Nucleic Acid Testing

In NAT, nucleic acid is extracted from donor plasma, and an amplification step such as reverse-transcription polymerase chain reaction (RT-PCR), polymerase chain reaction (PCR) or transcription-mediated amplification (TMA) is used to amplify and detect microbial genetic sequences. Testing is usually done on 'minipools' of 6–24 donor samples. Single-donor testing may be considered to

enhance sensitivity. Single-sample testing is required for resolution of a reactive pool to determine which donor sample contains the microbial target. Testing may be done individually for each agent or in a multiplex assay to identify several agents (human immunodeficiency virus [HIV], HBV and HCV) in a single reaction. Newer, completely automated platforms have reduced operational complexity.

NAT reduces the window period when donors may be infectious but have negative serological testing results. Window periods using serological assays are estimated as 15 days for HIV, 59 days for HCV, 67 days for HBV; and 9.5 days for HIV, eight days for HCV and 38 days for HBV using NAT. The utility of NAT depends on the incidence of infections in donors, which determines the likelihood of serological window-period donations [13,14]. In countries such as the UK, Canada and the USA, where incidence rates are extremely low, the NAT yield, i.e. the number of infectious donations detected by NAT alone, is extremely low [5], while yield is higher in countries such as South Africa, which has a higher incidence of HIV. The changing epizoology of arthropod-borne infections, such as West Nile virus (WNV), has led to mandated testing in some jurisdictions. For WNV, single-donor instead of minipool NAT may be used at the height of an epidemic in a given community.

Screening Tests and Donor–Recipient Matching

Table 23.1 lists screening tests used in different countries [7]. Some tests are mandatory and used to screen all donations; others are used on selected higher-risk donors (e.g. risk of past residence or travel) or to meet needs of specific patient populations. For some pathogens, such as malaria, a combination of donor deferrals and donor testing is used [15]. Cytomegalovirus (CMV) antibody testing may be done on some donors to provide CMV seronegative components for patients at risk of severe infection. Plasma used for manufacture may require additional NAT for agents including hepatitis A virus (HAV), hepatitis E virus (HEV) and parvovirus B19, which are not as effectively inactivated by the fractionation process. In France, HEV NAT is undertaken on a fraction of plasmapheresis donations destined for high-risk patients (transplant recipients, patients with chronic liver disease or immunodeficiency). The US Food and Drug Administration (FDA) requires blood operators in states with a high incidence of babesia to screen all donations for *Babesia* [16]. Selective testing on specific populations or donations destined for specific blood products (e.g. granulocytes) may be also undertaken for human T-cell lymphotropic virus-1/2 (HTLV-1/2) and Chagas [17,18]. Outside of Europe, Australia and North America, blood screening practices for transmissible diseases are variable (Table 23.2).

Decision Making for Test Implementation

The decision to implement a screening test varies between countries and depends on factors including the frequency of the infectious disease in donors, available testing technologies and morbidity associated with transfusion-transmitted infection. For example, in the USA all donations are screened in minipools for Zika virus, while in Canada a 21-day deferral is used to defer donors and reduce transfusion-transmission risk [19,20]. The Alliance of Blood Operators' risk-based decision-making framework may assist blood operators in determining whether to implement a screening test.

Quality Framework and Operational Issues

The microbiological and blood group safety of the blood supply depends on the input and interaction of a number of quality and operational factors. A formal quality management system is an important part of ensuring that blood donation testing is adequately performed. According to the Commission Directive 2005/62/EC (30 September 2005) of the European Parliament, a quality system for blood establishments should

Table 23.1 Screening tests on blood donations in five countries as of 2016.

Infection	Test	USA	Canada	France	UK	Australia
HIV-1, -2	HIV antibody	√	√	√	√	√
	HIV NAT	√	√	√	√	√
HCV	HCV antibody	√	√	√	√	√
	HCV NAT	√	√	√	√	√
HBV	HBV surface antigen (HBsAg)	√	√	√	√	√
	HBV NAT	√	√	√	√	√
	Antibody to HBV core antigen (anti-HBc)	√	√	√	Selective	Selective
HTLV-1/2	HTLV antibody	√	√	See note[1]	See note[2]	See note[3]
Syphilis	*Treponema* antibody	√[4]	√	√	√	√
Malaria	Malarial antibody	No	No	Selective	Selective	Selective
Chagas disease	*Trypanosoma cruzi* antibody	See note[5]	Selective	Selective	Selective	No
West Nile virus	WNV NAT	√	Seasonal	See note[6]	Selective	No[7]

√ Indicates testing on all donations.
[1] HTLV-1/2 (antibody) only new donors (mainland and Réunion) and all donations in Guadeloupe or Martinique.
[2] HTLV-1/2 screening on donors not previously tested and donations used to make non-leuco-depleted components (e.g. granulocytes).
[3] HTLV-1/2 is on first-time donors and granulocyte donors only.
[4] Either treponemal or non-treponemal is allowed for screening.
[5] One-time testing alone, without donor questioning for history of Chagas disease.
[6] When and where epidemic (mainland, as well as overseas): WNV, Dengue (as well as Chikungunya and Zika) NAT testing (if not and if localised occurrences: interruption of donations in the involved area(s)).
[7] Except for research studies.
HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HTLV, human T-cell lymphotropic virus; NAT, nucleic acid testing; WNV, West Nile virus.
Source: Adapted from O'Brien et al. 2012 [7].

include elements of continuous quality improvement, quality management and quality assurance. Topics addressed should include quality control, non-conformance and self-inspection, documentation, collection, testing and processing blood component recall, external/internal auditing, personnel, premises, equipment, storage, distribution and contract management. Directives are designed to allow for member states to have flexibility in establishing regulatory mechanisms and specific solutions.

Regulatory bodies play a key role in protecting the safety and quality of the blood supply. In the UK, the Medicines and Healthcare products Regulatory Authority's (MHRA) responsibilities include ensuring that blood components for transfusion meet standards for safety, quality and efficacy; promoting international standards and harmonisation; and promoting safer and more effective use of blood products. In the USA, there are both FDA regulations and extensive requirements from professional accrediting organisations such as the Association for the Advancement of Blood and Biotherapies (AABB) regarding quality requirements. Safety and quality are enhanced by ensuring the following:

- Testing is performed by staff trained in approved standard operating procedures (SOPs).
- Laboratories and staff are involved in quality assessment activities.
- Document control systems are in place to ensure that only current procedures are used, and changes documented and approved.
- Errors that occur are logged using a quality incident report (QIR) system, which requires corrective and preventive action to be taken.

Table 23.2 Distribution of donor screening for pathogens outside of Europe, Australia and North America: HIV-1/2, HBV and HCV, number and percentage of countries in region.

	HIV		HBV		HCV	
Region	**Ab or Ab + Ag**	**Ab + NAT or Ab + Ag + NAT**	**HBSAg +/– routine or selective anti-HBc**	**NAT + serology**	**Ab +/–Ag**	**NAT +/– serology**
Africa (n=46)	44 (96%)	2 (4%)	44 (96%)	2 (4%)	44 (96%)	2 (4%)
Eastern Mediterranean (n=20)	14 (70%)	5 (25%)	14 (70%)	5 (25%)	14 (70%)	5 (25%)
South-east Asia (n=11)	9 (82%)	2 (18%)	9 (82%)	2 (18%)	9 (82%)	2 (18%)
Western Pacific* (n=25)	18 (72%)	7 (28%)	18 (72%)	7 (28%)	17 (68%)	7 (28%)

* No HCV test: n = 1; 1/25 (4%).
Ab, antibody; Ag, antigen, HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NAT, nucleic acid testing.

- Blood operators partner with hospitals for notification of adverse events or recalls.

Most blood operators have surveillance programmes for monitoring rates of transmissible infections in donors, while haemovigilance schemes in place in several countries to monitor transmission of transfusion-transmissible agents. The reporting of serious adverse events resulting from transfusion is an essential component of blood safety.

Key Points

1) Every blood donation is tested for ABO and D and the presence of red cell antibodies.
2) Phenotyping for other red cell antigens may be important for transfusion support of particular recipient groups.
3) Laboratory screening tests form the core of the process to identify infected blood components prior to transfusion.
4) Reduction of transfusion-transmission risks may also rely on donor-deferral strategies.
5) Processes must be in place to communicate infectious disease marker results and other unexpected results of possible consequence to donors.
6) Both immunoassays and nucleic acid testing are used to identify possibly infectious units.
7) A quality framework is important for the accuracy of all laboratory testing as well as the safety, quality and efficacy of the blood supply.

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24 Production and Storage of Blood Components

Marissa Li[1], *Rebecca Cardigan*[2], *Stephen Thomas*[3] *and Ralph Vassallo*[4]

[1] *Cerus Corporation, Concord, CA, USA*
[2] *NHS Blood and Transplant, Cambridge, UK*
[3] *NHS Blood and Transplant, London, UK*
[4] *Vitalant, Scottsdale, AZ, USA*

Whole Blood and Its Processing to Components

Guidelines from the UK, Council of Europe, Health Canada, Association for the Advancement of Blood and Biotherapies (AABB) and the US Code of Federal Regulations (CFR) variously define a whole blood donation as 450–500 mL (±10%) of blood collected into citrate anticoagulant also containing phosphate and dextrose. The clinical indications for transfusion of whole blood are limited to intrauterine/neonatal exchange transfusion and, increasingly, massive transfusion. There is renewed interest in the use of whole blood in the prehospital and early hospital settings to provide a logistically simpler method for providing balanced resuscitation. However, the vast majority of collected whole blood is processed into components – red cells, plasma and platelet concentrates. Whole blood–derived plasma (sometimes called recovered plasma) is suitable for fractionation to plasma derivatives, freezing as transfusable plasma or further manufacture into cryoprecipitate and cryoprecipitate-depleted plasma.

Component production from whole blood consists of centrifugation to separate plasma and cellular material by size and density, followed by manual or automated transfer of components from the primary collection pack to storage packs. Collection and storage packs are manufactured as a single closed unit to maintain sterility. Whole blood donations from which platelets are to be harvested must be held and processed at 20–24 °C. For other donations, preprocessing storage and centrifugation of whole blood can be at either 20–24 °C or 1–6 °C. Some countries permit overnight holding of whole blood at 20–24 °C prior to component production, yielding components of acceptable quality, allowing the production of platelets from the majority of collections and obviating the need for multiple manufacturing shifts.

Collection of Components by Apheresis

Apheresis involves serial or continuous separation of the donor's blood into components inside disposable kits on specially designed equipment, with harvest of specific

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blood elements and reinfusion of the remainder [1]. Apheresis technology permits the collection of multiple transfusable doses of components from desired ABO- and RhD-specific groups. This is not possible with whole blood collections, which yield a single red cell, plasma and partial platelet dose of a given blood type. Therefore, the collection of two O+, O–, A– or B– red cells, two to five A or AB plasmas or two or three full-dose platelets may be obtained. The frequency of apheresis component donations is determined by the guidelines established in each country. Double red cell donation requires longer interdonation intervals (as well as higher haemoglobin cut-offs), while plateletpheresis allows collection of one to three adult doses per procedure as often as 24 times per year. Total allowable plasma loss varies by jurisdiction and donor blood volume, but is always less than 15 L per rolling calendar year.

Apheresis safety has been enhanced by the development of instruments with low extracorporeal volume and smaller, more portable machines have permitted collections at mobile sessions. Most plateletpheresis still occurs at fixed sites, since the enhanced efficiency of larger instruments results in greater platelet yields. Single donor-derived platelets (as opposed to pooled whole blood–derived units) may be required to reliably produce increments in patients with antibodies to human leucocyte or platelet antigens. Depending upon the diversity of the population and size of the country, 10–40% apheresis composition of the platelet supply is required to assure the product heterogeneity necessary for specialised support of alloimmunised recipients. While more expensive to produce, apheresis components require no further manufacture to produce an adult therapeutic dose, allowing blood centres to decrease the size of their component laboratories. This may accelerate the trend of 'near-donor' processing, which is slowly replacing traditional whole blood donation as a result of increasing pressures for type-specific component collections in some countries.

Regulations, Specifications and Quality Monitoring

Specifications for the key parameters of each component type are generally defined in a national guideline, such as those published by the UK Blood Transfusion Services, AABB and, in the USA, the CFR. The US Food and Drug Administration (FDA) and Health Canada, both charged with the oversight of blood safety, publish directives and guideline documents outlining good manufacturing practice. European guidelines published by the Council of Europe are not legally binding, but are intended to promote improvements in practice. However, in 2005, the European Union (EU) Directive 2002/98/EC, 'Setting Standards of Quality and Safety for the Collection, Testing, Processing, Storage and Distribution of Human Blood and Blood Components', became legally binding (with periodic subsequent amendments) in the UK as the Blood Safety and Quality Regulations (BSQR) 2005. In the UK, compliance of blood establishments with UK guidelines and the BSQR is regulated by the Medicines and Healthcare products Regulatory Authority (MHRA); for more details see Chapter 20.

Many countries sample a proportion of blood components for quality monitoring to assess compliance with set specifications. The proportion tested is usually determined by statistical process control, but is typically about 1% of components produced [2]. Statistical process control identifies systems that are capable of performing better and highlights trends towards poor performance at an early stage, so that corrective action can be put in place to address the problem.

Red Blood Cell Production and Storage

Red cells may be produced either from whole blood donations (Figure 24.1) or by apheresis (Table 24.1). For the vast majority of red cell components, an additive solution is

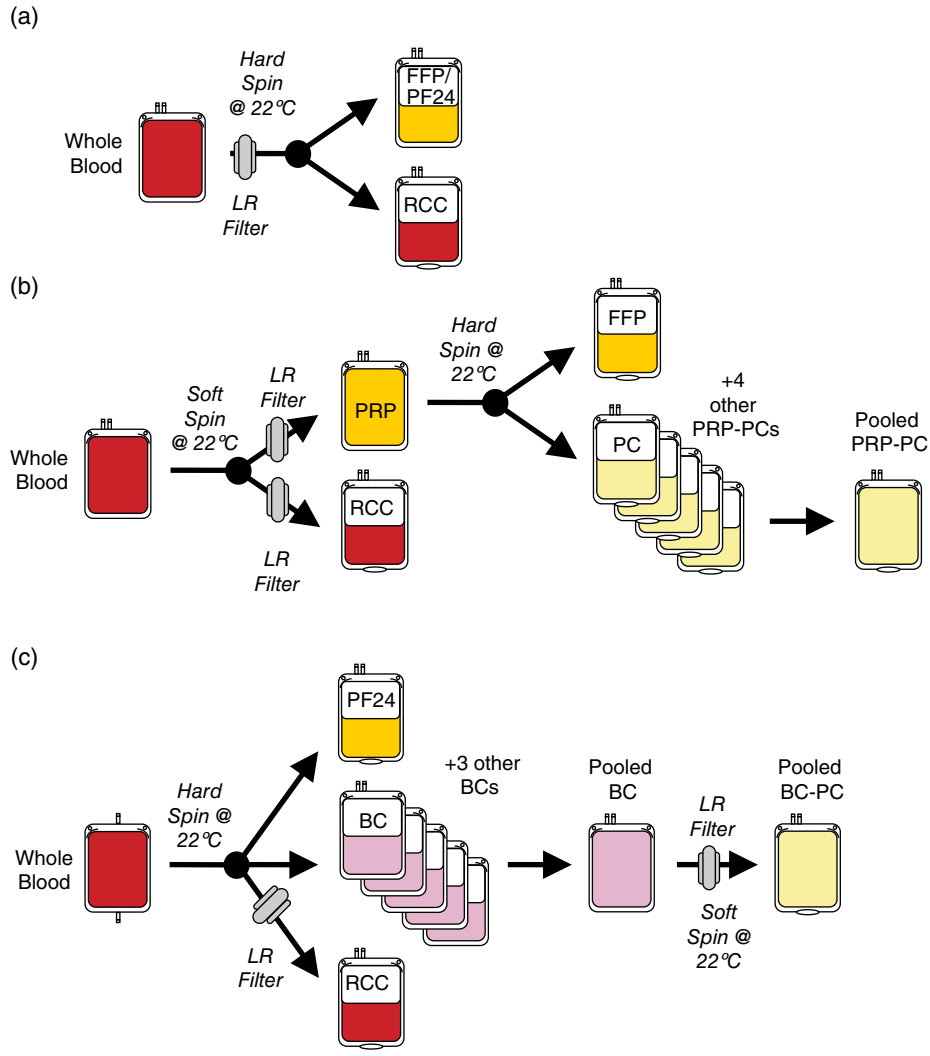

Figure 24.1 (a) Production of leucocyte-reduced (LR) red cell concentrates (RCC) and LR fresh frozen plasma (FFP) or plasma frozen within 24 hours of phlebotomy (PF24). (b) Production of LR RCC, LR FFP and platelet-rich plasma (PRP)-intermediate LR platelet concentrates (PC). (c) Production of LR RCCs, PF24 and buffy coat (BC)-intermediate LR PCs.

introduced following separation to achieve a haematocrit of 50–70% and extend storage from 21–28 days to 35–42 days. In the USA and Canada, red cells can be stored without additive solution with a 21–35-day shelf-life, depending upon the base anticoagulant solution in the collection set. Red cells in additive solution have a 35–42-day shelf-life, according to jurisdictional approval for specific additive solutions. Red cell storage temperatures start at 1–2° C, extending through 6° C, with allowance during transport up to 10 °C. To minimise the possibility of bacterial proliferation and maintain viability, red cells should be removed from refrigeration as little as possible.

The most important changes occurring during storage are progressive extracellular leakage of potassium and a decline in red cell recovery to 75–85% of transfused cells at end expiration. Red cells used for intrauterine transfusions (IUT) and exchange transfusion

Table 24.1 Specifications for red cell components.

	Volume		Haematocrit		Hb content			Haemolysis		WBC content (/LR unit)			Other	
Preparation	**CoE***	**UK†**	**CoE***	**AABB**	**CoE***	**UK†**	**AABB**	**CoE***	**UK†**	**CoE***	**UK**	**AABB**	**CoE***	**AABB**
Red cells (in CPD, CP2D or CPDA-1) ± LR	280 ± 50 mL; TBD for LR	280±60 mL for LR	65–75%	≤ 80%	≥ 40 g for LR	≥ 40 g for LR		< 0.8%	< 0.8%	< l× 10^6	> 90% < 1 × 10^6 and > 99% < 5 × 10^6	95% < 5 × 10^6		> 85% pre-LR recovery
Red cells, in AS (-1,-3, -5 or -7, SAGM, PAGGSM) ± LR	TBD	280±60 mL for LR; must be < 375 mL	50–70%		≥ 45 g; ≥ 40 g for LR	≥ 40 g for LR; must be ≥ 30 g		< 0.8%	< 0.8%	< l × 10^6	> 90% < 1 × 10^6 and > 99% < 5 × 10^6	95% < 5 × 10^6		> 85% pre-LR recovery
Red cells, buffy coat removed ± in AS	250 ± 50 mL; TBD in AS		65–75%; 50–70% in AS		≥ 45 g; ≥ 43 g in AS			< 0.8%		< 1.2 × 10^9				
Red cells, apheresis ± LR, ± AS	TBD		65–75%; 50–70% in AS		≥ 40 g		95% ≥ 50 g and mean 60 g; 95% > 42.5 g and mean 51 g for LR	< 0.8%	< 0.8%	< l × 10^6	> 90% < 1 × 10^6 and > 99% < 5 × 10^6	95% < 5 × 10^6		
Thawed reconstituted red cells, (thawed and washed), cryopreserved	> 185 mL	TBD	35–70%		≥ 36 g	≥ 36 g		< 0.2 g sup.‡ Hb/unit	< 2 g sup.‡ Hb/unit	< 0.1 × 10^9	> 90% < 1 × 10^6 and > 99% < 5 × 10^6	95% < 5 × 10^6	Max 20 mOsm/L over resuspending fluid; < 2 g sup.‡ Hb/unit	> 80% pre-glycerol recovery

* Minimum 90% of units must meet required value.
† Minimum 75% of units must meet required value.
‡ Unlike other measures of haemolysis expressed as % red blood cells haemolysed, after thawing and washing, this standard requires < 2 g of Hb in the unit's supernatant.
AS, additive solution; CoE, Council of Europe; CP2D, citrate phosphate double dextrose; CPD, citrate-phosphate-dextrose; CPDA-1, citrate-phosphate-dextrose-adenine; Hb, haemoglobin; LR, leucocyte reduced; PAGGSM, hosphate-adenine-glucose-guanosine-saline-mannitol; SAGM, saline-adenine-glucose-mannitol; TBD, to be defined (for the system used); WBC, white blood cell.

of neonates are normally stored or reconstituted in compatible plasma. Typically, clinicians will request freshly collected or washed units for potassium-sensitive patients to avoid levels as high as 95 mEq/L of supernatant (5–6 mEq per bag) at the end of storage. For patients who require red cells and have a history of severe or recurrent allergic reactions, or immunoglobulin A (IgA) deficiency with anti-IgA, red cells are washed and resuspended in saline or an approved additive solution. This removes up to 95% of plasma proteins and donor antigens to which patients have preformed antibodies. Automated and manual closed systems are available for cell washing that allow red cells to be stored for up to 14 days after washing instead of 24 hours if processed in an open system [3]. Red cells from donors with rare phenotypes or autologous units from patients with one rare or multiple common red cell alloantibodies, for whom provision of compatible donor blood is extremely difficult, can be stored frozen for 10 years or longer. Prior to transfusion, frozen red cells are thawed and washed (deglycerolised) to remove the cryoprotectant used to preserve them.

Platelet Production and Storage

Platelets may be separated from whole blood donations and subsequently pooled, or collected by apheresis (Table 24.2). Platelet production from whole blood requires two centrifugation steps that differ in their intermediate. In the UK, Europe and Canada, pooled buffy coats (BC) are generated by 'bottom and top' processing, while in the USA, platelet-rich plasma (PRP) is the intermediate (see Figure 24.1) [4]. BC and apheresis platelets yield similar posttransfusion increments, while PRP platelets tend to produce lower increments. This has been attributed to harsher centrifugation against a plastic surface and consequent increased

Table 24.2 Specifications for platelet components (90% of units must meet required values unless noted otherwise).

Preparation	Volume (CoE)	Platelet content	WBC content (/unit)	Expiry $pH_{22\,°C}$
Platelets (recovered, single unit) – PRP intermediate	> 40 mL per 0.6×10^{11} platelets	CoE: > 0.6×10^{11} AABB: ≥ 0.55×10^{11} (75% for LR)	CoE: < 0.2×10^{9} AABB: 95% < 0.83×10^{6} for LR	CoE: 100% > 6.4 AABB: ≥ 6.2
Platelets (recovered), pooled ± AS	> 40 mL per 0.6×10^{11} platelets	CoE: ≥ 2×10^{11} UK: 75% ≥ 2.4×10^{11}; 75% ≥ 2×10^{11} in 100% AS (24-hour expiration); all must be ≥ 1.6×10^{11}	CoE: < 0.3×10^{9} from BC, < 1×10^{9} from PRP, < 1×10^{6} for LR UK: < 1×10^{6} and > 99% < 5×10^{6} for LR AABB: 95% < 5×10^{6} for LR	CoE: 100% > 6.4 UK: 95% > 6.4 AABB: ≥ 6.2
Platelets, apheresis ± AS	> 40 mL per 0.6×10^{11} platelets	CoE: ≥ 2×10^{11} UK: 75% ≥ 2.4×10^{11}; 75% ≥ 2×10^{11} in 100% AS (24-hour expiration); all must be ≥ 1.6×10^{11} AABB: ≥ 3×10^{11}	CoE: < 1×10^{9}, < 1×10^{6} for LR UK: < 1×10^{6} and > 99% < 5×10^{6} for LR AABB: 95% < 5×10^{6} for LR	CoE: 100% > 6.4 UK: 95% ≥ 6.4 AABB: ≥ 6.2

AABB, Association for the Advancement of Blood and Biotherapies; AS, additive solution; BC, buffy coat; CoE, Council of Europe; LR, leucocyte reduced; PRP, platelet-rich plasma; WBC, white blood cell.

activation for PRP platelets compared to the softer red cell cushion against which BC platelets are concentrated. The significantly lower cost of pooled whole blood–derived platelets contrasts with their marginally greater risk of viral, parasitic and bacterial disease transmission and the inability to match alloimmunised recipients with a compatible full-dose apheresis unit. Leucocyte reduction (LR) by filtration or by in-line apheresis technology is easily accomplished. An adult therapeutic dose of platelets ($> 2.5 \times 10^{11}$) can be consistently manufactured from four or more whole blood donations. In contrast, with the appropriate selection of donors, 1–3 adult therapeutic doses ($2.5–11 \times 10^{11}$) can be harvested from a single donor during one apheresis collection procedure.

Platelets are stored under agitation at 20–24 °C for five days, which may be extended to seven days if an approved method to detect or inactivate bacterial pathogens is used. Cold-stored platelets were used in the 1960s and 1970s. The markedly decreased circulation times of these platelets because of cold-induced neoantigen formation and reticuloendothelial destruction led to their replacement with agitated 20–24 °C platelets [5]. Cold-stored platelets are an area of active study for limited use in bleeding patients due to their better immediate haemostatic properties compared with room temperature–stored units in platelet aggregometry and patient bleeding time studies. Currently, the use of frozen platelets stored in dimethyl sulfoxide (DMSO) is mainly restricted to military blood banks. However, studies are ongoing to assess the suitability of this product in the civilian setting [6].

With prestorage LR and modern storage plastics, platelets stored for seven days in plasma maintain their *in vitro* function, with 15–20% reductions in recovery compared with five-day stored platelets. During storage, platelets undergo a fall in pH due to accumulation of lactic acid, show increased surface expression of activation markers and lose their normal shape. Many different laboratory assays have been advocated to monitor development of the so-called platelet storage lesion, but few have been demonstrated to correlate with *in vivo* survival [7]. pH remains the only quantitative change that must be monitored routinely and must be above 6.2–6.4 at outdate. Visual inspection to look for the 'swirling' effect of discoid platelets has been recommended, but this is highly subjective and changes only when the platelets have been grossly damaged.

For patients with severe allergic reactions, usually due to plasma proteins, it is possible to 'wash' platelets by centrifugation and replacement of the plasma with saline or an additive solution. This results in the loss of > 20% of platelet number and function, but does ameliorate reaction rates far more than simple plasma volume reduction.

Platelet additive solutions (PAS) are available worldwide for apheresis platelets and, in some countries, whole blood–derived platelet pools [8]. These solutions contain sodium chloride, acetate, citrate, phosphate or gluconate buffers ± potassium and magnesium. Platelets stored in 65% PAS and 35% plasma are available in a number of countries and can be stored for 5–7 days. This strategy makes more plasma available for transfusion or fractionation, appears to reduce allergic reactions, but may result in lower platelet increments, depending on the additive used. Solutions incorporating glucose and bicarbonate require less plasma carry-over, which may further reduce plasma-based reactions.

Plasma Production and Storage

Fresh frozen plasma (FFP) from a whole blood donation must be separated and frozen as soon after collection as possible, within eight hours in the USA and Canada and preferably within six hours in continental Europe (Table 24.3). Usual unit volumes are 200–300 mL. FFP can also be derived from apheresis collections in 300–600 mL volumes. It is

Table 24.3 Specifications for plasma components.

Preparation Storage	CoE (95% of components) ≤ −18 to −25 °C: 3 mo., < −25 °C: 36 mo.	UK (75% of components) ≤ −18 to −25 °C: 3 mo., ≤ −25 °C: 36 mo.	AABB (100% of components) ≤ −18 °C: 12 mo.; FFP only: < −65 °C: 7 yr.
Fresh frozen plasma (FFP)	Platelets < 50×10^9/L RBC < 6×10^9/L WBC < 0.1×10^9/L, < 1×10^6/U if LR FVIII mean ≥ 0.7 IU/mL, ≥ 0.5 IU/mL if PR Fibrinogen ≥ 60% of original FFP after PR	Platelets < 30×10^9/L RBC < 6×10^9/L WBC < 1×10^6/U if LR FVIII mean ≥ 0.7 IU/mL (must be ≥ 0.3 IU/mL and 90% must have ≥ 0.5 IU/mL) Protein ≥ 50 g/L Volume 200–340 mL	
Plasma frozen within 24 hours after phlebotomy (± held at room temperature up to 24 hours)	Same as FFP	Same as FFP	
Liquid plasma	Not recognised	Provisional component	
Cryoprecipitate	FVIII ≥ 70 IU/unit Fibrinogen ≥ 140 mg/unit vWF ≥ 100 IU/unit Volume 30–40 mL	WBC < 1×10^6/unit if LR FVIII ≥ 70 IU/unit Fibrinogen ≥ 140 mg/unit	FVIII ≥ 80 IU/unit Fibrinogen ≥ 150 mg/unit
Pooled cryoprecipitate		WBC < 1×10^6/unit if LR FVIII ≥ 350 IU/unit Fibrinogen ≥ 700 mg/unit Volume 100–250 mL	FVIII in pool ≥ 80 IU/unit Fibrinogen in pool ≥ 150 mg/unit
Plasma, cryoprecipitate depleted		Platelets < 30×10^9/L RBC < 6×10^9/L WBC < 1×10^6/U if LR	

AABB, Association for the Advancement of Blood and Biotherapies; CoE, Council of Europe; FVIII, Factor VIII; LR, leucocyte reduced; PR, pathogen reduction; RBC, red blood cell; vWF, von Willebrand factor; WBC, white blood cell.

commonly used as a source of multiple coagulation factor replacement for massive transfusion, disseminated intravascular coagulation, warfarin-induced bleeding and liver disease. It can also be used for plasma exchange in patients with thrombotic thrombocytopenic purpura (TTP), or serve as a single source of one or more deficient factors for which no concentrates are available. The permitted shelf-life (three months to seven years) depends on the storage temperature (≤ −18 to ≤ −65 °C). In Europe, FFP must be monitored for levels of factor VIII. FFP is thawed in a protective overwrap in a water bath, a purpose-designed microwave oven or dry heat source. Once thawed, FFP should be used as soon as possible since the levels of labile coagulation factors decline during further storage. Most countries permit thawed plasma to be stored refrigerated for at least one day and up to five days in some if it is relabelled as 'thawed plasma.' Thawed plasma has been demonstrated to contain lower levels of the labile coagulation proteins FV and FVIII, but this does not appear to significantly impact its clinical efficacy [9].

Frozen plasma (plasma frozen within 24 hours after phlebotomy in the USA) is

obtained from whole blood or by apheresis and frozen within 24 hours after collection. Its storage requirements, therapeutic efficacy and clinical use are the same as for FFP. In North America this component is labelled as a product different from FFP, whereas in Europe this is not the case.

Plasma, cryoprecipitate reduced, is a by-product of cryoprecipitated antihaemophilic factor production and is deficient in FVIII, von Willebrand factor (vWF), fibrinogen, FXIII and fibronectin. This component is stored at the same temperature and duration as FFP. Once thawed, it must be used within 24 hours or, as allowed in some countries, stored refrigerated for up to five days after relabelling as thawed plasma, cryoprecipitate reduced. Its sole use is as a replacement fluid during plasma exchange for TTP.

In the USA, liquid plasma is collected from whole blood, stored between 1 and 6 °C and expires in 26–40 days, depending upon the base anticoagulant into which it is collected. Use in North America is generally limited to 14–21 days because of progressive factor loss. The clinical indication for liquid plasma is limited to initial treatment of massively transfused patients with life-threatening haemorrhage. As a never-frozen product with viable lymphocytes, to prevent transfusion-associated graft-versus-host disease (TA-GVHD) irradiation is recommended.

Cryoprecipitate Production and Storage

Cryoprecipitate is manufactured by slowly thawing single units of FFP at 1–6 °C. Cryoprotein precipitates of factors VIII and XIII, vWF, fibrinogen and fibronectin are concentrated 2–9-fold compared with plasma (see Table 24.3) [10]. Thus, it is important to remember that cryoprecipitate is not simply a concentrated form of FFP, as many pro- and anticoagulant factors are not found in cryoprecipitate. In North America, each single unit of cryoprecipitate must contain ≥ 80 IU and ≥ 150 mg fibrinogen in approximately 5–20 mL of plasma; ≥ 70 IU and ≥140 mg respectively (generally in 30–40 mL) are required in the UK and Europe. Cryoprecipitate can be stored for 1–3 years, depending on temperature and local regulations. Thawed cryoprecipitate has a shelf-life of 4–6 hours, depending upon jurisdiction and open or closed system processing. A novel pathogen-reduced cryoprecipitated fibrinogen complex (PRCFC) has been approved in the USA, indicated to treat bleeding associated with fibrinogen deficiency. Thawed PRCFC has a five-day shelf-life stored at room temperature [11]. An alternative method of extending the shelf-life of cryoprecipitate under investigation is prolonged refrigeration following thawing to reduce the risk of bacterial proliferation, followed by a brief rewarming at 37 °C [12]. Although originally developed for factor VIII deficiency (haemophilia A), most cryoprecipitate is now prescribed to treat acquired hypofibrinogenaemia, usually in the context of massive transfusion, disseminated intravascular coagulation or liver disease. An adult dose of 5–10 bags is generally indicated once the fibrinogen level falls below 1.0–1.5 g/L.

Some countries pool five or more bags of cryoprecipitate to facilitate its administration. An alternative product would be a virus-inactivated fibrinogen concentrate, but in many countries this is only licensed for congenital qualitative or quantitative defects. Clinical studies demonstrating efficacy of either cryoprecipitate or fibrinogen concentrate for acquired deficiency are accruing, particularly in the setting of massive haemorrhage resuscitation protocols and orthopaedic or cardiovascular surgery with high risk of bleeding [13].

Granulocyte Production and Storage

The transfusion of granulocyte concentrates is uncommon. They are presently indicated only for severely neutropenic patients (count $< 0.5 \times 10^9$/L) who have a reasonable expec-

tation for return of marrow function and for bacterial or fungal infections refractory to appropriate antimicrobial therapy. Granulocytes are primarily collected by apheresis, with buffy coat separation from whole blood as an alternative source. Most regulatory agencies require an adult dose of $\geq 1 \times 10^{10}$ granulocytes, which is usually infused daily. To achieve such doses, apheresis donors' peripheral counts are increased with steroids ± granulocyte colony-stimulating factor (G-CSF). Unstimulated apheresis granulocyte collections often do not result in a minimally acceptable adult dose. G-CSF mobilisation with ~5 μg/kg G-CSF plus oral dexamethasone 8 mg (which further elevates counts and blunts some of the side effects of G-CSF) 12–24 hours prior to apheresis results in collections of 6–8 × 10^{10} granulocytes, a dose sufficient to elevate patients' circulating counts. At present, use of G-CSF for granulocyte collection is permitted in volunteer donors unrelated to the patient in the USA, but not in other countries. Apheresis donors are also exposed to a sedimenting agent (hetastarch or pentastarch) during apheresis, which decreases product red cell contamination.

A large but underpowered randomised controlled trial of the efficacy of high-dose granulocyte transfusion in infected neutropenic patients concluded that granulocytes had no significant impact on survival or improvement of infection at 42 days after randomisation [14]. However, *post hoc* analysis showed that patients receiving higher doses ($> 0.6 \times 10^9$ cells/kg) had a survival advantage compared to those receiving lower doses, with a trend towards improvement over untreated controls.

Some European countries transfuse buffy coats as a source of granulocytes. A dose of 1×10^{10} can be achieved from 10 buffy coats. A pooled granulocyte component made from 10 buffy coats has been developed in the UK and its safety assessed in clinical studies [15].

Granulocytes should be transfused as soon as possible after collection due to their 24-hour shelf-life and the onset of significant functional deficits within 6 hours of collection. As a consequence of the short transfusion timeframe, infectious disease test results are not usually available prior to release, so many collectors require prequalified donors. Granulocytes must be irradiated to prevent TA-GVHD, and never leucocyte reduced. Granulocytes should be kept at 20–24 °C without agitation. Because of red cell contamination, a crossmatch should be performed. Substantial numbers of platelets are also present in granulocytes, usually $> 2.5 \times 10^{11}$.

Component Modifications

Many countries have implemented or are progressing towards universal LR of blood components, since passenger leucocytes have no known therapeutic effect but do confer risk for certain reactions and infections. Prestorage LR is usually carried out at the blood centre with filters during component separation of whole blood, or by in-process removal of leucocytes during plateletpheresis. Proven benefits of LR include reduced transmission of human T-lymphotropic virus (HTLV)-I/II and herpes viruses (including cytomegalovirus, Epstein–Barr virus and human herpes virus 8), and decreased risk of febrile non-haemolytic transfusion reactions and human leucocyte antigen (HLA) alloimmunisation [16]. While theoretically reducing the risk of prion transmission, LR does not eliminate infectivity. A small amount of cellular loss (< 10%) accompanies filtration LR. European requirements are the most stringent, requiring demonstration of $< 1 \times 10^6$ leucocytes per component (a greater than 3-log reduction). The AABB requires $< 5 \times 10^6$ per component or, in the case of unpooled whole blood–derived platelets, one-sixth of this amount. Various percentages of units required to meet specifications and the statistical sampling requirements to demonstrate compliance exist across national regulatory agencies. LR failures do occur randomly in small percentages of units as a result of poor apheresis separation, filter manufacturing

defects or, more commonly, donor-related filtration issues like sickle cell trait. Erythrocyte sickling has been shown to block some filters or create channels between fibres, allowing leucocytes to pass more efficiently.

Irradiation of leucocyte-containing/contaminated components with 1500–3000 cGy inactivates donor lymphocytes whose proliferation in recipients with compromised immune systems results in TA-GVHD [17]. GVHD has been observed after transfusion from blood relatives or HLA-selected units, as well as in fetal and neonatal recipients of intrauterine transfusions. Granulocyte transfusions can and should be irradiated, since granulocytes are relatively radio-resistant, unlike contaminating lymphocytes. Unlike platelets that are also resistant, red cells sustain membrane damage, which requires a shortening of shelf-life. This is generally the earlier of 28 days after irradiation or the original expiry date, but nuances exist for various products and radiation doses.

Pathogen-inactivation technologies have been approved for plasma and platelets in Europe and the USA and are under development for red cells. Four systems for producing pathogen-inactivated plasma are available [18]. Three are suitable for single donor plasma: visible light + methylene blue (MB) and UV light + amotosalen or riboflavin. The fourth, solvent-detergent (SD) treatment, is applied to plasma pools. All methods generally offer a $\geq$ 4-log reduction (range 2–7) of viruses, bacteria and parasites, but all are associated with 20–30% loss of clotting factors. Amotosalen and MB are removed by adsorption prior to transfusion (the latter not in all European countries), while riboflavin is not. SD treatment of ABO-identical pools of hundreds of donors' plasma destroys lipid-enveloped viruses and many bacteria and parasites, but does not affect non-enveloped viruses. Accordingly, hepatitis A and parvovirus B19 testing is performed to select units for pooling and SD treatment. Some SD processes introduce a prion-reduction step, and in the UK as a primary variant Creutzfeldt-Jakob disease (vCJD) risk-reduction method, until late 2019 donor plasma was sourced from countries at low risk for vCJD for individuals born on or after 1 January 1996. In 2021, the UK also removed the ban on UK-sourced blood plasma for the manufacture of immunoglobulins. In the USA, enthusiasm for pathogen-reduced plasma, various preparations of which have been FDA approved since 1998, has been low due to cost and improvements in viral and parasitic safety resulting from progress in testing.

An FDA mandate to improve platelet bacterial safety has increased enthusiasm for pathogen reduction in the USA, despite significantly higher costs, adding the USA to the list of countries concerned about emerging transfusion-transmitted infectious diseases as well as bacterial contamination. Three technologies exist: use of amotosalen + UV-A (INTERCEPT™, Cerus Corporation, Concord, CA, USA), riboflavin + UV-A/B (Mirasol™, Terumo BCT, Lakewood, CO, USA) and UV-C alone (THERAFLEX UV-Platelets™, MacoPharma, Turcoing, France). The UV-C technology is CE-marked but not in wide use in any country, while the former two have gained various jurisdictional approvals and are in use for platelets stored in either plasma or additive solution. All three technologies produce UV-induced nucleic acid damage, inactivating DNA and RNA in pathogens and the leucocytes that cause GVHD. There is variable protein damage, which results in reduction of posttransfusion increments, with variably observed increases in transfusion frequency. Nevertheless, pathogen-reduced platelets are effective in terminating and preventing haemorrhage. Pathogen inactivation of platelets obviates the need for irradiation and cytomegalovirus (CMV) testing, and in some jurisdictions permits a seven-day shelf-life, which reduces wastage. However, until a red cell pathogen-inactivation system is also available, some of these benefits cannot be fully realised.

Red cells present a challenge for pathogen-inactivation technologies due to the high degree of light absorption by haemoglobin. A second-generation Cerus system employs S-303, an acridine nitrogen mustard alkylator,

which remains in phase III trials following the suspension of first-generation trials due to neoantigen formation and red cell alloimmunisation. The Mirasol system can be used on whole blood, albeit relying upon higher energies and treatment time, which results in lower log reduction of some pathogens and significantly reduced red cell viability. It is likely that it will be several years before either system is licensed for routine use. A prion filter capable of a 3–4-log reduction in red cells has been available since 2007, but cost-effectiveness has been a barrier to wider implementation [19].

KEY POINTS

1) Whole blood is limited to certain clinical settings, therefore most whole blood is separated into its components for transfusion (red cells, plasma and platelets).
2) Blood components can be produced from whole blood donations or collected directly from the donor by apheresis technology.
3) Blood component storage conditions vary as a function of the production method, ideal temperature for maximal function and the intrinsic life span of the cells or proteins in the product.
4) Systems are now available in all jurisdictions to inactivate pathogens in plasma or platelet components prior to storage.

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25 Blood Transfusion in Hospitals

Zoe K. McQuilten[1], Erica M. Wood[1], Mark H. Yazer[2] and Michael F. Murphy[3]

[1] *Transfusion Research Unit, Department of Epidemiology and Preventive Medicine, Monash University; Department of Clinical Haematology, Monash Health, Melbourne, Australia*
[2] *Department of Pathology, University of Pittsburgh, Pittsburgh, PA; Centralized Transfusion Service, Pittsburgh, PA, USA; Department of Pathology, Tel Aviv University, Tel Aviv, Israel; Department of Clinical Immunology, University of Southern Denmark, Odense, Denmark*
[3] *Radcliffe Department of Medicine, University of Oxford; NHS Blood and Transplant, John Radcliffe Hospital; Department of Haematology, Oxford University Hospitals, Oxford, UK*

The aim of transfusion practice is to provide 'the right blood to the right patient at the right time for the right reason'. In the framework of transfusion safety, the focus is on ensuring that, when it is clinically indicated, patients receive transfusion support to meet their particular needs, in a safe, timely and cost-efficient manner, with incidents and adverse reactions recognised, managed and reported effectively and with measures in place to prevent occurrence or recurrence. This chapter discusses the roles and responsibilities of the main participants in transfusion in hospitals (Table 25.1), and highlights bedside and administrative practices that have been implemented around the world to improve transfusion safety.

Transfusion safety is an organisation-wide concern. A focus on safety needs to cascade from the highest levels of hospital management right through to the staff managing the bedside processes. Hospital administrators at all levels set the tone by establishing an organisational safety culture and a reporting system for errors and near-misses that encourages reporting in order to correct ambiguous or inefficient processes.

The hospital transfusion committee is a vital component in ensuring patient safety. By encouraging open discussion among participants about adoption of best practices in all aspects of transfusion medicine, dangerous 'workarounds', or ways to circumvent established processes, can be discovered and solutions developed to support staff to adhere to safe practices. The transfusion committee also needs to audit clinical transfusion practice to ensure that agreed evidence-based thresholds set for transfusion of blood products are being followed, adverse events are investigated, needed transfusions are provided in a timely way, and at the same time ensuring that under-transfusion – the withholding of a necessary transfusion – is not occurring.

Enormous investments have been made to reduce the risks of transfusion-transmitted infections but, until recently, there has been much less investment in improving hospital systems required for clinical practice. Consequently, evidence of progress in reducing procedural risks and improving the safety of hospital transfusion practice is slower to accumulate. Some interventions, such as the

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Table 25.1 Process steps, risks managed, persons responsible and resources involved in hospital transfusion practice.

Process	Risk being managed	Responsible								Resources
		Medical	Nursing	Phlebotomists	Porters	Laboratory staff	Transfusion team*	HTC	Quality system†	
Education/ training ↓	Poor clinical decision making Inadequate awareness of risks/ benefits of transfusions Errors in every step of process									Policies/procedures to ensure all staff involved in transfusion process are appropriately trained and competency is assessed
Clinical assessment/ decision to transfuse ↓	Inappropriate transfusions Undertransfusion									Local transfusion/PBM guidelines Disease-specific treatment guidelines Access to alternatives to allogeneic transfusion (e.g. cell salvage)
Inform patient, obtain consent ↓	Patient insufficiently informed about risks, alternatives Failure to take account of patient's personal beliefs/views									Standardised patient information Patient informed consent form
Select product and quantity ↓	Mistransfusion (e.g. not meeting specific needs) Blood wastage									Guidelines for selection of appropriate products (e.g. irradiated blood products) Maximum Surgical Blood Ordering Schedules for elective surgery

(*Continued*)

Table 25.1 (Continued)

Process	Risk being managed	Responsible								Resources
		Medical	Nursing	Phlebotomists	Porters	Laboratory staff	Transfusion team*	HTC	Quality system†	
Order product ↓	Incomplete or incorrect patient information Failure to request special components									Standard request form (paper/electronic) Documentation of indication for transfusion (ideally with clinical decision support)
Positive patient identification ↓	Patient misidentification									Systems and policies for patient identification, including unknown patients and maintenance of unique identifiers if e.g. wristband removed
Pretransfusion sample collection ↓	Patient misidentification Delays and rework									Standard request forms (paper/electronic) Hospital sample labelling policy
Crossmatching, selection of product(s) ↓	Incorrect units issued, e.g. in worst case ABO incompatible, or units issued not meeting patient's special needs Blood components not available Lack of audit trail									SOPs for crossmatching, storage, transportation of blood products Retention and storage of patients' blood samples Documentation to allow traceability of products

Delivery to clinical area ↓	Incorrect units collected Lack of audit trail Delays and their consequences									Hospital policy for blood collection
Identity check, blood group/ special requirement ↓	Incorrect unit transfused, including correct ABO/RhD									Hospital policy and procedure for correct patient identification and for the administration of blood
Administration of product ↓	Prevention and timely management of transfusion reactions									Hospital policy and procedures for monitoring of patients during transfusion
Documentation ↓	Lack of audit trail									Hospital policy and procedures for documentation for traceability of blood products
Monitor/ respond/ investigate/report adverse event ↓	Transfusion errors, and transfusion reactions and their management									SOP for documenting, investigating and reporting of errors and adverse transfusion reactions Review of these events and learning from them to prevent their recurrence
Audit	System weaknesses and inefficiencies, opportunities for improvement Blood wastage Patient harm									Hospital policy and procedures for the monitoring, reporting and investigation of adverse events Regular review of blood use and transfusion practice and feedback to clinical teams and individual clinicians Indicators for monitoring quality and safety of transfusion process

*Hospital transfusion team – see text in chapter.
†Refers to hospital governance quality systems in place.
HTC, hospital transfusion committee; PBM, patient blood management; SOP, standard operating procedure.

practice of a bedside ABO group check before transfusion, requiring an ABO group confirmation on a second sample before group-specific blood products are issued to recipients without a historical ABO group on file [1], or the use of physical barriers to transfusion, such as a code to link the patient's wristband to the pretransfusion sample and unlock the designated unit of blood from secure storage, are intrinsically attractive. For a variety of reasons, they have been difficult to implement widely [2]. Data to support the effectiveness of many procedural interventions are still limited and serious (and often preventable) adverse events continue to be reported. However, where haemovigilance programmes have been able to highlight these issues and their causes, and action has been taken to address them, progress has been demonstrated [3]. For example, bedside electronic processes for patient identification and confirmation that the correct sample is taken for compatibility testing and that the correct unit of blood is transfused have reduced errors and the risk of wrong transfusions [4,5]; these processes will be described in greater detail later in the chapter.

Effective quality frameworks are required to minimise transfusion risks and ensure that the supply of donated blood is managed effectively, minimising unnecessary use and reducing wastage at every step of the process. These in turn require a patient-centred approach to transfusion, committed leadership and adequate resources.

What Does Hospital Transfusion Practice Encompass?

Hospital transfusion practice encompasses all of the processes required to ensure that transfusion of blood and blood components is appropriate to patients' clinical needs, is timely and is delivered safely. This includes transfusions in community settings such as aged care or hospice settings, or in patients' homes, where this is in place.

The process, as outlined in Table 25.1, begins with the assessment of the patient and decision to transfuse and includes a series of interconnected steps, including prescribing, pretransfusion testing, selection and delivery of blood products, administration and monitoring, evaluation of the response to the transfusion and auditing. The transfusion process is complex, as was recently illustrated by a study conducted to estimate the true cost of red cell transfusion. This study developed process maps to describe the step-by-step outline of the entire transfusion pathway, which involved over 600 individual activities [6].

Positive patient identification, good documentation and excellent communication underpin all stages of the transfusion process [7]. Specialists in all branches of medicine and surgery are involved in transfusion, and engagement, cooperation and coordination are required by staff and patients to manage the complex, interacting sequences of the process. Of course, the staff who collect the pretransfusion testing samples and who ultimately administer the transfusions are at the forefront of patient safety and must recognise their responsibilities for correct patient identification and sample labelling practices, and perform these tasks with great care and accuracy. The hospital transfusion medicine service contributes to patient safety by providing clinical leadership; by having qualified personnel available to answer transfusion-related questions from staff, patients and families; by investigating suspected adverse events; and by advocating for the best interest of patients when non-evidence-based practice is detected. Thus the responsibility for safe transfusions is found at all levels of a hospital's administrative, medical and nursing staff, bringing complementary skills and expertise and working collaboratively to ensure the final goal is met for every patient, every time.

Lessons for Hospital Blood Transfusion Practice from Haemovigilance Programmes

What happens when the culture of safety breaks down and an error happens? Mistransfusion, or 'wrong blood' events, i.e. administering a unit of blood that either does not meet the patient's special needs or is intended for another recipient, can have serious consequences, including severe haemolysis due to antigen–antibody incompatibility, and is a well-recognised cause of mortality and morbidity. Human errors leading to mistransfusion can occur at any step in the process, and usually result from failures to comply with clerical or technical procedures, or systems that are either poorly constructed or not understood (see Table 25.2 for examples). Multiple errors are frequently involved in these cases: the so-called Swiss cheese model of error, whereby several layers of safeguards are bypassed, leading to the mistransfusion. Some miscollection errors can be detected during the bedside check at the time of administering blood, and this remains a final opportunity to prevent mistransfusion. It has been observed that as many as 1 in 19 000 red cell units are given erroneously and 1 in 33 000 will involve ABO-incompatible units [8].

In many countries, blood for transfusion is not safe, sufficient or reliably available. In these settings, haemorrhage remains a major direct cause of mortality. However, even in modern healthcare settings with adequate blood supplies, occasionally patients die from transfusion complications [3,9], or from lack of adequate transfusion support. Some instances of undertransfusion are due to patient refusal to accept transfusion support, or failure by physicians to recognise and respond to clinical manifestations of bleeding. However, others can be attributed to either

Table 25.2 Examples of some errors and other problems in the transfusion process, and their potential outcomes.

Problem	Potential outcome
Unnecessary prescription	Patient subjected to unnecessary risks, including transfusion-associated circulatory overload Blood component wastage
Prescribed components do not meet patient special requirements	Transfusion complications (e.g. transfusion-associated graft-versus-host disease, alloimmunisation)
Blood not stored in controlled environment	Blood component wastage Transfusion complications (e.g. risk of bacterial growth)
Pretransfusion samples taken from incorrect patient Sample transposition or other laboratory errors Incorrect unit of blood collected and/or administered	Mistransfusion and potential for immediate haemolytic reaction from ABO-incompatible transfusion Potential for RhD alloimmunisation
Insensitive techniques in pretransfusion testing	Potential for acute and delayed haemolytic transfusion reactions or sepsis, particularly from platelets
Poor management of blood stocks	Blood component wastage Inappropriate overuse of group O red cells and potential for consequent shortages of that group
Delay in emergency provision of blood components	Patient morbidity/mortality due to bleeding, hypoxia or abnormal haemostasis

lack of knowledge of transfusion protocols or failures of communication within, and between, clinical teams. At the other extreme, patients are frequently overtransfused, and transfusion-associated circulatory overload (TACO) is increasingly recognised as the most frequent serious adverse event (see Chapter 10) [10]. Inappropriate transfusions are a waste of blood components, which are a scarce resource, and put patients at unnecessary risk

All of the processes and elements outlined in Table 25.1 are essential for safe transfusion practice in hospitals, as reflected in haemovigilance reports, which often highlight where systems have failed. Key messages from recent Serious Hazards of Transfusion (SHOT) reports and other haemovigilance programmes have included [3,11,12]:

- The importance of leadership and fostering a strong and effective safety culture.
- Accurate patient identification as fundamental to patient safety.
- The vital role of clinical and laboratory staff training in the fundamentals of transfusion to ensure safe, high-quality patient-centred care.
- Learning from near-misses as a key element to finding and controlling risks before actual harm results.
- The importance of simple, clear and easy-to-follow standard operating procedures, as many incidents are due to a failure to follow correct procedures.
- The potential of information technology to improve transfusion safety by prompting staff through the correct steps of each process and therefore minimising human factors leading to errors.
- Appropriate staffing levels in all areas involved in transfusion, and staff not permitted to undertake tasks for which they have not received training and been competency assessed.
- Systematic and thorough investigation of incidents to allow identification of systems-based corrective and preventive actions.

Key Features of Hospital Transfusion Governance

Most countries require blood centres or services (termed 'blood establishments' in Europe and 'blood collectors' in the USA) and hospital transfusion laboratories to maintain robust quality systems to ensure good practice, including meeting national or regional standards for good manufacturing, laboratory and/or clinical practice (also see Chapter 20). These requirements are typically overseen by national regulatory authorities and/or professional authorities to ensure compliance. For example, the UK Blood Safety and Quality Regulations [7] outline requirements for quality management in transfusion laboratories, including staff training, process validation, documentation, storage and handling, traceability and reporting of adverse events. However, these regulations have typically not extended to transfusion practice in clinical areas, and other measures are still needed to ensure that processes and systems that influence the quality and governance of clinical transfusion practice at the hospital level are optimised and working as expected. Recommendations for transfusion practice outside the blood transfusion laboratory are derived from clinical experience along with lessons from haemovigilance, external quality assessment (EQA) schemes, and the evidence base for the clinical use of blood. These are translated into policies, standards and guidelines by government agencies and professional groups, who in turn assess implementation and compliance and promote best practice through training, education and communication.

In England, the Care Quality Commission regulates healthcare providers. Many of the national standards for governance and risk assessment are applicable to blood transfusion, including those relating to patient engagement, informed consent, staff training and competency assessment, participation in audit and other quality improvement activities and reporting of incidents. Specifically, the National Patient Safety Agency in England

in 2006 issued a safety notice requiring competency-based training and assessment for all staff involved in blood transfusion, a pretransfusion bedside identity check that requires staff to match the blood unit with the recipient's wristband (rather than the compatibility form or case notes), and a formal risk assessment for use of any alternative means of confirming patient identity.

Similar expectations apply in other countries. For example, the Australian Commission on Safety and Quality in Healthcare standard on clinical transfusion practice is part of national safety and quality standards [13] and outlines requirements against which hospitals are assessed for accreditation. In the USA, authorities from state health departments through to national regulators like the Joint Commission on Accreditation of Healthcare Organizations (JCAHO) and the Food and Drug Administration (FDA), as well as private institutions such as the AABB (Association for the Advancement of Blood and Biotherapies, formerly known as the American Association of Blood Banks), are involved in assessing and regulating transfusion practice.

At an institutional level, executive management is responsible for implementation of standards and policies, including initial and ongoing staff training and appraisal, and for performing and monitoring clinical audits and other quality system activities.

Hospital Transfusion Committees

Hospital transfusion committees (HTCs) are focal points for overseeing transfusion practice at the institutional level. Their roles are outlined in documents such as English NHS 'Better Blood Transfusion' Health Service Circulars, and statements from regulatory bodies like the US JCAHO.

HTCs go by various names, including blood management committees if they also have oversight of broader patient blood management (PBM) activities. They are essential components of clinical governance, so they must be incorporated into hospital frameworks for clinical governance, performance and risk management, and report findings and activities in a timely and meaningful way, accompanied by recommendations for action where appropriate. HTCs have the remit to promote best practice, review clinical transfusion practice, monitor performance of the hospital transfusion service, participate in regional or national initiatives and communicate with local patient representative groups as appropriate (Table 25.3) [14].

Suggested membership for HTCs is shown in Box 25.1. A chairperson with understanding and experience of transfusion practice should be appointed by hospital senior management. Ideally, the chairperson should not be the medical specialist responsible for the hospital transfusion service, who could be perceived to have a vested interest. It is very helpful to include representatives from medical and surgical specialties that order blood components (such as from anaesthesia, surgery, critical care, obstetrics and the emergency department), nursing staff from hospital units that are regularly involved in administering blood transfusions (such as haematology/oncology) and from the PBM programme, to contribute their clinical perspectives.

To be effective and to deliver on their objectives, HTCs require support from dedicated hospital transfusion teams (see below), at a minimum consisting of a medical specialist, transfusion practitioner(s) [15] and blood transfusion laboratory scientist/manager. Other necessary resources include information technology (IT) and clerical support to facilitate regular meetings, data retrieval and audit.

Working Together to Improve the Transfusion Process

The Multidisciplinary Hospital Transfusion Team

This team supports the HTC and works collaboratively to identify and take action on areas for improvement in hospital practice.

Table 25.3 Activities of the hospital transfusion committee.

Area or activity	Example
Policies and procedures	Develop and promulgate policies and procedures, including for: • Clinical indication and decision to transfuse • Establishing and enforcing transfusion thresholds • Informed consent process • Collection of samples for compatibility testing, including patient identification and specimen labelling requirements • Transfusion administration and monitoring • Indications for specialised components (e.g. irradiated, cytomegalovirus [CMV]-seronegative, phenotype matched) • Maximum Surgical Blood Ordering Schedule (MSBOS) • Blood conservation strategies, including use of cell salvage and pharmacological agents • Management of patients who decline transfusion • Management of adverse reactions
Education, training and assessment	Develop strategy for education, training and assessment of all staff involved in transfusion Monitor implementation and results of education and training activities Develop and/or promulgate information/materials for patients
Audit, monitoring and review	Develop annual audit plans and monitor performance Review adverse event reports Conduct incident, 'near-miss' and sentinel event reviews Oversee traceability and record-keeping obligations
System performance	Review: • Blood component availability, utilisation, crossmatch : transfusion ratios and wastage rates • Activation of massive transfusion protocol, use of uncrossmatched emergency red cell stocks • Performance of institutional transfusion laboratory and blood service ('blood establishment') • Participation in regional and national audit, transfusion practice improvement and haemovigilance programme activities • Participation in external quality assessment activities Oversee hospital and laboratory accreditation activities relating to transfusion Contingency and disaster planning

In addition to the members outlined above (transfusion medicine specialist, biomedical scientist and transfusion practitioner), other contributors may include registrars (fellows) or residents undertaking specialist training in transfusion medicine; personnel with quality management responsibilities in clinical or laboratory areas; or representatives of PBM, cell salvage or other related clinical programmes; and students, either as regular or occasional invited participants.

Activities depend on organisational needs, and typically focus on problem solving and quality improvement, such as adverse event and incident evaluation, haemovigilance reporting, transfusion policy and procedure development and audit planning and analysis. The group can coordinate transfusion communication, education and research activities and HTC support. Close links with other clinical practice improvement and PBM activities, such as preoperative anaemia management, are important.

Box 25.1 Suggested membership for hospital transfusion committee.

- Representatives of all major clinical blood users, including junior medical staff.
- Specialist haematologist/pathologist with responsibility for transfusion.
- Hospital blood transfusion laboratory senior scientist/manager.
- Specialist practitioner(s) of transfusion.
- Senior nursing representative.
- Representatives from hospital management and clinical risk management.
- Local blood centre medical specialist (*ex officio*).
- Other co-opted representatives as required, e.g. from medical records, portering staff, clinical audit, training or pharmacy.
- In many settings, participation by a patient representative is encouraged, and in some cases is expected, and may be a requirement of hospital accreditation.

To be effective, the hospital transfusion team should meet frequently (daily, weekly or at least monthly) and the HTC should meet as often as it needs to achieve its aims – many hospitals have monthly or quarterly HTC meetings. Team meetings also provide teaching opportunities from interesting clinical cases, and may suggest research and quality projects for further development by the group or others.

The Role of the Patient and Informed Consent

'Patient-centred care is a dimension of safety and quality' [13]. The concept of patient-centred care recognises the essential role of patients in participating in their own care. Patients are increasingly educated and informed, and can (and should, wherever possible) play important roles in care planning and delivery. For example, individual patients can participate at all stages of the transfusion process, from discussions about what alternatives may be available and the need for any special requirements such as irradiated or antigen-matched units, to ensuring correct identification at the time of pretransfusion sample collection or bedside administration, and early recognition of adverse events [16].

Patient groups can also play important roles in hospital practice on behalf of their members. For example, community groups representing patients with haemoglobinopathies, bleeding disorders or other major conditions that may require transfusion support, or Jehovah's Witnesses, can provide important input to developing educational materials and ensure these are available, up to date and culturally appropriate.

Communication between patients and clinical staff is essential for healthcare planning and delivery, including for procedures like transfusion that carry potential benefits but also important hazards.

Hospital-informed consent processes for transfusion have historically focused on documentation of staff informing patients about the risks of blood components and fractionated products, with little ability to demonstrate that this information is understood by the intended recipients or that the information meets the needs of patients and their families. More recently, the focus has shifted to a process of communication that aims to ensure that patients receive meaningful information relevant to their individual circumstances and in a way that they can understand and use. Definitions and expectations vary between countries and institutions, but broadly speaking, for transfusion, patients should understand why transfusion might be necessary, what it would involve and what alternatives are available, including the potential benefits and risks of the various options under consideration.

Staff conducting the conversation should have sufficient knowledge of transfusion practice and current risk estimates of infectious and non-infectious hazards to provide accurate information. Ready access to written guidance prepared for clinical staff or patients can facilitate these interactions, as many staff and patients have very limited understanding of the real risks of transfusion.

The HTC can be useful in preparing such documents. Many national blood services and practice improvement programmes also provide summaries, which are periodically updated with local data. Sufficient time also needs to be allocated for the discussion so that patient questions can be addressed.

For a range of reasons, some patients will not accept transfusion, or will only accept very limited transfusion options [17]. Hospitals must have policies and procedures in place to manage these situations, in line with the principles of respect for patient autonomy and working within organisational and regulatory requirements. For all patients, including those who decline any or some transfusion support, open discussion, clear communication and documentation of options and agreed plans are essential [18].

Administration of Blood and Blood Components and Management of the Transfused Patient

The transfusion process is complex and involves multiple steps – each of which carries the potential for error and delay.

Errors occurring at blood sampling, collection and administration can lead to patient misidentification and mistransfusion. Prescription errors, however, lead either to failure to provide special components to meet the recipient's special needs or to transfusions that are unnecessary or inappropriate and carry the potential for complications. For example, TACO has occurred when transfusions have been given on the basis of a spuriously low haemoglobin value resulting from samples taken from intravenous ('drip') arms or measured by gas analysers, or a clerical error where another patient's results are reported. Fatal errors have also occurred in prescribing the wrong volume to transfuse or the wrong rate of transfusion. Failure to monitor transfused patients, particularly in the first 15 minutes of receiving each unit, can lead to life-threatening reactions being overlooked and delays in resuscitation.

Hospitals should have written procedures to cover all these steps, against which relevant staff are trained and regularly assessed, and which are readily available for reference at the bedside. Clinical responsibilities, actions, documents, potential errors and some of their consequences are outlined in Table 25.1. Prescription charts, donation numbers of components and batch/lot numbers of fractionated plasma products issued and transfused, nursing observations and recipient vital signs related to the transfusion should be kept as permanent clinical records. Regulatory and accreditation authorities require a complete audit trail of blood to the patient's bedside. Many hospitals comply with this requirement by returning signed and dated compatibility forms or compatibility labels to the transfusion laboratory. Electronic methods are increasingly replacing hard-copy documents, with associated improvements in reliability and efficiency.

Technologies to Reduce Patient Misidentification Errors in Administering Blood

The manual process for patient identification is shown in Figure 25.1. Bedside handheld devices, barcoded staff identity badges, barcoded printed wristbands for patients and portable printers taken to the bedside for sample tube labels provide means for improving the accuracy of patient identification and thus transfusion safety [4,19]. For example, at sample collection, the identity of the phlebotomist and patient can be established by scanning their respective identity cards or wristbands, and barcoded labels generated at the bedside containing the patient's full identification details can be attached to the sample tube at the time and place where it is collected. In the laboratory, allocated units are labelled with a compatibility label including a barcode incorporating both the patient's unique identification details and the unit number.

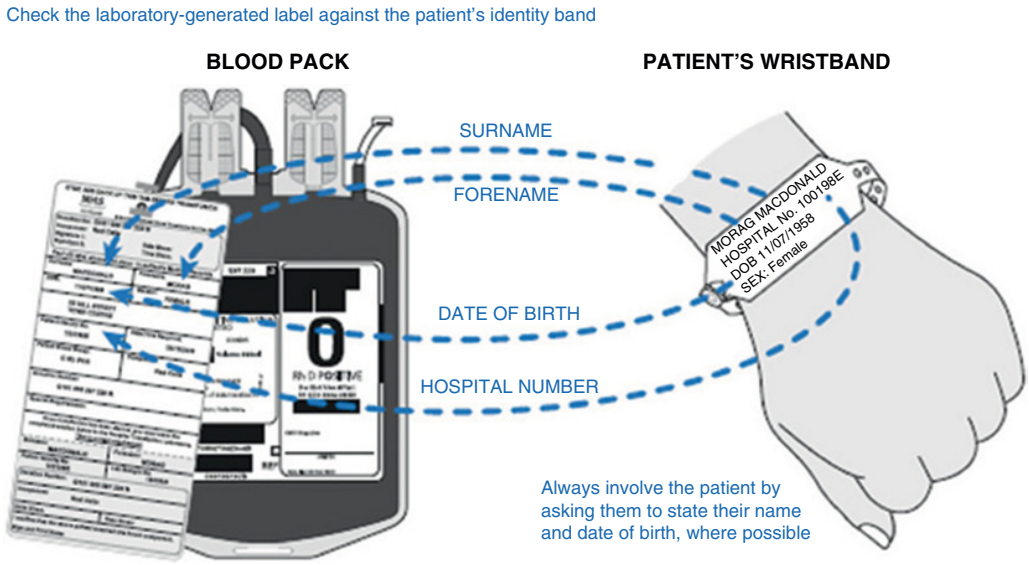

Figure 25.1 How to perform an identity check between the patient and a blood component. *Source:* Handbook of Transfusion Medicine, 5th edn, Stationery Office, UK.

At the time of pretransfusion checking, staff are prompted by a handheld device to scan their own identification barcode, and the barcodes on the patient wristband, the compatibility label and the unit number on the blood component (Figure 25.2). The handheld device prompts the staff to verbally check the identity of the (conscious) patient and the barcode scans confirm that the unit is correct for the patient. The user and transfusion laboratory are alerted if there is a mismatch. The device also provides prompts to check for special requirements, pretransfusion observations and the unit expiry date. Documentation of each step is transmitted to the laboratory information system to confirm the traceability of the unit, and to facilitate assessment of the competency of staff in safe transfusion practice.

Electronic bedside systems can be linked to similar systems controlling release of blood from remote blood refrigerators to provide full electronic process control, and to facilitate electronically controlled remote issue (see later in this chapter and [20]).

Electronic systems for blood transfusion are increasingly being implemented [4,5], although further studies are needed to confirm their cost-effectiveness. The acceptance of their use would be even greater if they were integrated with other processes requiring patient identification, such as medication administration.

Influencing Clinical Practice

Potential factors influencing transfusion practice and decision making include:

- Physician knowledge and perception based on clinical experience and review of the available literature.
- Peer pressure and feedback.
- Effectiveness of hospital governance frameworks.
- Educational prompts and directions (e.g. through online ordering system settings) at the time of decision making.
- Patient knowledge and preferences [16].
- Financial pressures or incentives.
- Public and political perceptions and fear of litigation.
- Availability of blood products.

Improving transfusion practice within a hospital community requires a planned,

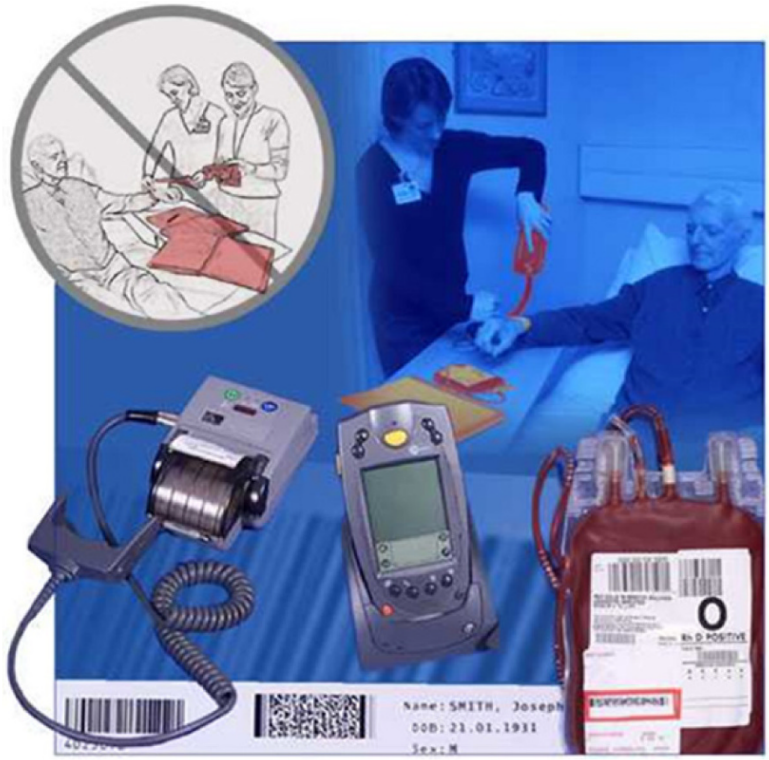

Figure 25.2 Bedside checking using an electronic system. The traditional method of pretransfusion bedside checking requires two nurses and checks of multiple items of written documentation. With barcode technology, a handheld computer reads a barcode on the patient wristband containing full patient details. The handheld computer checks that the patient details on the wristband barcode match those on the barcode (in the red box) on the compatibility label attached to the unit after pretransfusion testing. This barcode also contains the unique number of the unit, and is matched with the barcode number of the unit (top left of the bag) to ensure that the blood transfusion laboratory has attached the right compatibility label. *Source:* Reproduced with permission from John Wiley & Sons.

consistent approach, endorsed and implemented through clinical governance frameworks, supported over time and monitored for effect. In this endeavour, the HTC can be very useful in disseminating the latest evidence-based information and in auditing and ensuring prescriber compliance with hospital policies and procedures.

Guidelines, Algorithms and Protocols

Guidelines are systematically developed statements to assist practitioner and patient decision making about appropriate healthcare for specific clinical circumstances. A list of websites with some examples of guidelines is included at the end of this chapter. There are examples of changes in transfusion practice following publication of national PBM guidelines [21]; however, promulgation of national guidelines rarely leads to change without local implementation and dissemination strategies, and these require time and resources.

Developing an institutional strategy to implement guidelines is a useful opportunity to gain ownership and participation. For example, educational opportunities arise from examining the evidence basis for the guidelines, and dissent and other local barriers to

implementation, such as limited staff or IT resources, or effects on laboratory turnaround times can be identified and resolved.

Institutions should adopt recommendations from authoritative professional guidelines and from well-designed clinical trials (see Chapter 47). The data and recommendations should be carefully reviewed in light of the need for any customisation for local use. This may involve separating guidelines into sections and/or incorporating some recommendations into other local protocols for specific conditions, such as plasma guidelines incorporated into protocols for management of disseminated intravascular coagulation and massive haemorrhage. These local documents should be incorporated into transfusion policies and disseminated, with training, for all involved staff. A multidisciplinary approach to designing the local guidelines and thresholds allows for the input of the product users, so that their concerns are addressed.

Experience in other medical fields has demonstrated that embedding guideline recommendations into materials used during decision-making and administration processes can significantly improve compliance. Examples include:

- Listing clinical indications for special blood components on transfusion request forms or electronic request screens.
- Using electronic warning systems to alert prescribers when, based on laboratory values, planned transfusions do not meet guidelines (see below).
- Listing, on specific transfusion observation charts, actions to be taken in the event of reactions.
- Detailing checks to be made on the compatibility form prior to administering blood.

Intraoperative algorithms for the use of platelets and plasma to correct microvascular bleeding during and after cardiac bypass surgery have also been successful in reducing inappropriate use of these components, especially when combined with near-patient testing and rapid availability of results [22,23].

Clinical Audit and Feedback

Clinical audit and feedback is a quality improvement process that seeks to improve patient care and clinical outcomes, through systematic review of care against explicit criteria or standards, followed by the implementation of change. Analysis of audit findings can lead to recommendations for improvements when deficiencies or non-guideline-based practices are identified, in turn generating cycles where feedback and clarification of hospital policies lead to improved practice.

Audit and feedback is widely used as a strategy to improve clinical practice within quality improvement systems, and has been shown to lead to potentially important improvements in practice. A systematic review of randomised trials of audit and feedback, albeit with few trials specific to transfusion, has shown that the effectiveness of audit and feedback may depend on baseline performance and how the feedback is provided – including the source of feedback, the regularity of feedback, explicit goals and specific action plans [24].

Audits can be conducted retrospectively or concurrently. Retrospective transfusion audits are often performed under the auspices of the HTC. Some regulatory agencies require a certain percentage of all transfusions to be reviewed by the HTC and those felt to have been administered without reasonable justification brought to the committee's attention. If, from available data, a transfusion is felt to be egregious, further information should be requested from the responsible physician. If the explanation is inadequate or if the physician fails to reply, other steps, such as letters to department chairs, can also be taken. Advantages of this type of review are that communications from the HTC carry additional weight, and they can be educational tools to inform physicians of institutional protocols. The main disadvantages are the limited number of transfusion episodes that can be manually audited and, because audits are performed after the event, educational opportunities are lost if the staff who ordered the transfusion cannot be located or cannot recall

the event. Retrospective audits also cannot influence clinical practice for the episodes being audited (but might influence prescribing behaviour in the future). Availability of electronic medical records, coupled with use of artificial intelligence (AI) and machine learning techniques, offers the opportunity to review much larger data sets with fewer requirements for human resources.

Audits performed concurrently with blood component ordering, but before product issue, can take several forms. A simple example involves transfusion laboratory staff comparing component orders with hospital guidelines; if criteria are not met, the ordering physician is contacted, the reasons for ordering the transfusion discussed and plans established. Intervention by transfusion medicine physicians has been demonstrated to be effective in reducing unnecessary transfusions [25]. Audits of this type have been criticised for potentially causing delay in providing necessary products, although they would also prevent unnecessary transfusions before they were administered. Significant time, effort and good communication are required to make these audits effective.

Another approach to concurrent audit involves automation to warn clinicians at the time of ordering that the transfusion might not be necessary. Where a hospital uses computerised order entry, and institutional guidelines are in place, warnings can appear on the screen when physicians try to order blood for patients whose laboratory values suggest that transfusion is not indicated. Figure 25.3 demonstrates the response where a physician attempts to order red cells for a patient whose latest haemoglobin value is above the threshold set by the HTC. The warning appears, giving the physician the option of either cancelling the order or proceeding, depending on the patient's current clinical situation (which might not be accurately reflected in a historical laboratory value). This approach, combined with regular feedback to clinical teams about their compliance with agreed blood count thresholds, has been successfully implemented in Oxford [26]. The experience of the University of Pittsburgh Medical Center (UPMC) large multihospital healthcare system is that about 10–15% of the orders in which an alert appears are cancelled. This contributes to a reduction in unnecessary blood product transfusions and provides education around evidence-based transfusion practice. The ability to track individual physicians and hospital locations that generate the greatest number of warnings also supports the provision of focused education.

Many countries have regional or national clinical audit programmes, with participation

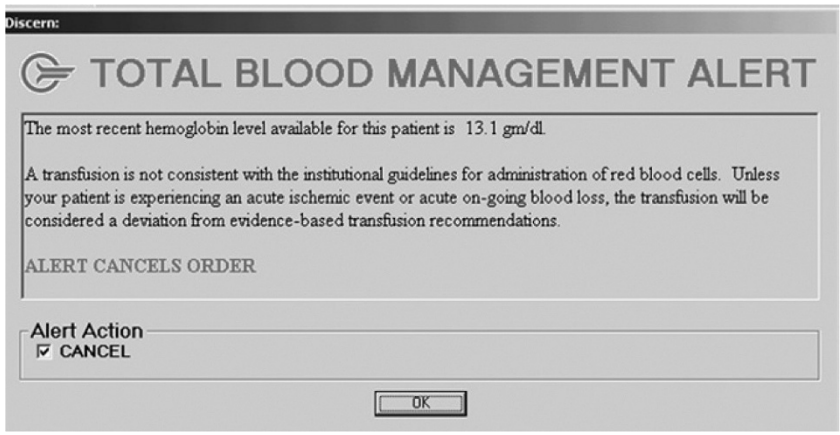

Figure 25.3 Warning message displayed when a physician at a University of Pittsburgh Medical Center (UPMC, Pittsburgh, PA) hospital attempts to order red cells using the computerised order entry system for a patient whose most recent haemoglobin value is in excess of the institutional guidelines.

being either voluntary or, increasingly, mandated by accreditation or governmental agencies. Participation provides opportunities to benchmark performance between similar institutions, and to promote engagement in practice improvement activities more broadly. The UK national audit programme and several of the practice improvement collaboratives in Australia have made their audit tools available to invite collaboration, comparisons of practice and sharing of resources.

Surveys

Many activities that fall under an 'audit' banner are not comparing practice with a standard, but are monitoring or surveying practice. These activities, many of which can be quantified, often provide information and baseline data that can lead to the development of quality or performance indicators. Trend analysis, or comparison of organisations or blood users with each other, is a powerful means of exerting peer pressure and influencing practice (benchmarking, as above).

Performance indicators can be applied to the following:

- *Clinical and laboratory practice issues*: for example the proportion of medical records with evidence of patients having received written pretransfusion information in a language understood by the patient and/or family member; percentage of primary hip or knee arthroplasties requiring allogeneic transfusion; proportion of patients receiving platelets after coronary artery bypass grafting; red cell use by surgical procedure (by surgeon or unit); or percentage of anaemic patients being investigated, correctly diagnosed and managed appropriately to minimise unnecessary transfusions.
- *Process issues*: percentage of mislabelled samples received in the laboratory; patient wristband errors; numbers of units crossmatched to units transfused (C : T) ratio; hospital blood wastage; percentage of group O red cells used.

National Schemes

Many countries now have regional or national schemes to monitor transfusion practice and promote practice improvement. These may be voluntary or mandatory, and institutions may be anonymous or identified. The programmes can be used to influence policy at national and local level, and to educate clinicians. Examples include the following:

- *Haemovigilance programmes*: the UK SHOT scheme is a voluntary system for collecting data on serious transfusion adverse events and near-misses. It produces annual reports with recommendations. Many other regional and national examples exist, and experiences presented in these reports have been very valuable in identifying areas for improvement. A national haemovigilance programme in the USA with voluntary hospital participation was launched in 2010 as a joint effort between the AABB and the Centers for Disease Control (see Chapter 21).
- *EQA schemes*: these programmes periodically provide clinical material to be tested by transfusion laboratories. Results are returned for analysis and collated reports disseminated to participants.
- *Utilisation and wastage schemes*: initiatives such as UK Blood Stocks Management and Australian BloodNet collate and publish details of blood stock inventory and wastage, and allow participants to benchmark against comparable hospitals.

Public and Political Perceptions and Fear of Litigation

Transfusion-transmitted human immunodeficiency virus (HIV) led to substantial reductions in allogeneic red cell use in many countries after 1982. These declines are even more significant considering population growth and changing demographics (e.g. ageing) during this period. Over the same interval, autologous donations increased greatly.

Some physicians were sued when transfused patients contracted HIV and the transfusions had not been clinically indicated.

The potential for transfusion-transmitted variant Creutzfeldt–Jakob disease (vCJD) was one of the concerns that led the UK Department of Health in 1998 to require that all hospitals should have HTCs, implement good transfusion practice and explore the feasibility of cell salvage. Universal leucocyte reduction of blood was introduced in the UK in 1999 as a further preventive measure for vCJD. This resulted in a significant increase in the price of blood, which was an additional encouragement for hospitals to implement more judicious approaches and use of alternatives to transfusion. As a consequence, red cell use in the UK has decreased by over 30% over the last 20 years, despite an increase in the volume and complexity of clinical care over this period.

Recent international focus on patient-centred care and PBM (see Chapter 37), partly arising from risk and cost concerns as described above, has simultaneously driven active and collaborative activities, involving patients, clinicians (general practitioners and hospital staff), hospital management and government health authority engagement in active and collaborative processes to promote alternatives to transfusion, for example by better management of anaemia and minimising blood loss.

Local Investigation and Feedback Following 'Near-Misses' and Serious Adverse Events

SHOT defines a 'near-miss' as an error or deviation from standard procedures or policies that is discovered before the start of the transfusion and that could have led to a wrong transfusion or a reaction in a recipient if transfusion had taken place [27]. These events are defined as any untoward occurrences associated with the collection, testing, processing, storage and distribution of blood or blood components, which might lead to death or life-threatening, disabling or incapacitating conditions for patients, or which result in, or prolong, hospitalisation or morbidity. Systematic root cause analyses of these incidents provide opportunities to detect and understand system and process weaknesses and take corrective action to minimise recurrence. Typical weaknesses identified through root cause analyses include inadequate training; human factors such as fatigue, misconceptions and ignorance of relevant policies; environmental factors such as distractions or interruptions, time pressures or access to equipment and IT support; and defective or risky processes.

Sample errors, most importantly those where the tube is labelled with the intended patient's details but contains blood from another patient, 'wrong blood in tube' (WBIT) events, are some of the most common detectable errors reported to haemovigilance programmes. These inevitably arise as a result of failures to systematically and positively identify the patient at the bedside [28]. However, investigations almost always uncover other contributing factors, which need to be understood and addressed:

- Failure to positively identify the patient. Healthcare workers have often not been trained in and are unfamiliar with hospital policies and procedures, or policies and procedures may be inconsistently implemented – for example, requiring inpatients but not outpatients to wear wristbands during treatment, including transfusion; some staff may also perceive this activity as unimportant or as suggesting that they have an inadequate knowledge of patients under their care. This may be compounded where 'agency' or temporary staff are working under unfamiliar conditions.
- Reduced junior doctors' hours and shift patterns of those involved in direct patient management, and inadequate communication and documentation, leading to unfamiliarity with patients.

- Admission and discharge practices, which may lead to patients having samples taken for pretransfusion testing before case notes are available or wristbands applied, leading to the potential for misidentification.

Exposure to avoidable patient morbidity or fatality often triggers patient, clinical and management awareness of transfusion hazards and can instigate procedural changes. Corrective action should involve counselling and educating individuals who failed to comply with procedures, but focusing on addressing the important, underlying system issues identified above, and supporting patients and staff in what are often traumatic situations [29].

Education and Continuing Professional Development

Education of all individuals in the transfusion process has traditionally been difficult, but UK experience shows it to be achievable when made an integral part of mandatory hospital training programmes and subject to external inspection. However, it requires considerable dedicated resources, a flexible and pragmatic approach to accommodate shift patterns and staff turnover, and availability of staff, including temporary staff and those who work 'after hours'. Observational competency assessment is more readily achieved with the help of clinical 'champions'. Training and knowledge-based assessments can be facilitated by web-based programmes (such as the e-learning modules of the Australian BloodSafe programme, for which over 500 000 staff nationally and internationally have registered), which also permit management oversight of participation.

Education is an essential component of strategies to gain clinician compliance with procedures and guidelines and to modify practice. Educational interventions are more successful when they are interactive, focused on a specific objective and directed at groups of individuals with reflections on their own practice. Continuing professional development schemes for the various groups of staff involved in transfusion encourages knowledge acquisition with documentation (typically via participant portfolios) of accredited activity in educational, professional and vocational areas.

Centralisation of Transfusion Services

The medical and patient safety benefits of a centralised transfusion service (CTS) vary depending on its organisation. The CTS in Pittsburgh, PA, USA (city population 306 000; catchment population 2.1 million) operates as follows. The main blood supplier, Vitalant, delivers blood products to a central laboratory. This centrally located facility also houses the red cell reference laboratory and performs most of the automated, batched pretransfusion testing. The laboratory then distributes products to over 25 CTS-networked hospitals in a 'hub-and-spoke' manner. Each hospital has an on-site transfusion laboratory, and is staffed and stocked with products in accordance with the acuity of patients treated and volume of transfusions performed. Each hospital laboratory performs routine pretransfusion testing and basic immunohaematology, thawing of plasma and cryoprecipitate, and some platelet pooling and leucocyte reduction (most is performed centrally).

Perhaps the most important patient safety benefit of a CTS is the ability to access patient records between different hospital sites. Since patients can visit different hospitals within the network, recipient immunohaematology and component modification requirements are available electronically at each hospital's blood transfusion laboratory, reducing the need for reinvestigation and ensuring that any special component modifications are fulfilled for each patient whenever and wherever transfusion support is required. Having records of recipient historical ABO groups provides additional opportunities to detect

WBIT errors. In 16 cases where recipient historical ABO groups on file at the Pittsburgh CTS did not match the ABO group of specimens submitted for pretransfusion testing, 6/16 were detected based on a historical ABO group that had been previously collected at a different hospital [30]. Requiring a second ABO group to be performed on a separate specimen before ABO-specific red cells are issued to recipients without an historical ABO group on file would achieve the same end, but requires considerable effort to implement. A recent study of two CTS systems in the USA demonstrated that some sickle cell disease patients with clinically significant antibodies were transfused at many different hospitals. Without the benefit of the CTS's recipient database, haemolytic reactions could have occurred, as in many cases these patients' antibody screens were negative at the time the transfusion was ordered [31].

Other advantages of a CTS include availability of transfusion medicine expertise for community hospitals without experts on staff. CTS transfusion physicians participate on HTCs of all networked hospitals, supporting rapid implementation of evidence-based practice and benchmarking. Consolidating technical expertise into one reference immunohaematology laboratory permits rapid and expert service provision. There are also numerous opportunities for cost savings, through greater efficiency from technical and non-technical employees, economies of scale and use of automation. Blood supplier logistics are greatly simplified by delivery to one central location, and lower inventory levels can be supported due to the ability to circulate blood products between hospitals to reduce wastage.

Maximum Surgical Blood Ordering Schedule

When more blood than is required is crossmatched and set aside for a patient, then it is unavailable for other patients and the chance is increased that it will expire before being used. Policies such as the use of a Maximum Surgical Blood Ordering Schedule (MSBOS) are helpful in preventing unnecessary expiry of blood, as well as preventing unnecessary blood draws for types and screens that are unlikely to result in transfusions. An example of an MSBOS for general surgery outlining what is routinely required before the case begins is shown in Table 25.4. The MSBOS is prepared taking into account the likelihood of transfusion and the response time for having blood available, following an immediate spin crossmatch or electronic issue. An MSBOS reduces the workload of unnecessary crossmatching and issuing of blood, and can improve stock management and reduce wastage.

The successful implementation of an MSBOS depends on all parties agreeing to the schedule, education of blood prescribers, confidence of senior staff that there is a robust system for accessing blood promptly when there is unexpected blood loss, and ability to override the schedule when there are reasons indicating that greater blood loss will occur. The schedule is constructed by the following:

- Analysing each surgical procedure in terms of C : T ratio.
- Routinely managing procedures with a C:T ratio greater than, for example, 1.8 (i.e. a low probability of transfusion) with a group and screen, and issuing blood only when there is a need for transfusion.
- Allocating an agreed number of units for procedures with a C : T ratio of less than 1.8.

An overall C : T ratio of 1.5 for elective surgery is achievable when the laboratory is centrally issuing blood in accordance with the MSBOS. However, lower ratios are possible with use of electronic crossmatch and/or remote electronic issue from blood refrigerators in theatre suites.

In addition to reducing the number of allocated, crossmatched red cells for specific surgical patients, which increases the number of

Table 25.4 Example of a Maximum Surgical Blood Order Schedule (general surgery) [32].

Procedure	Requirements (G&S = group and screen)
Abdomino-perineal resection	G&S
Amputation (below or above knee)	G&S
Anterior resection	G&S
Appendectomy	Nil
Apronectomy (mini-abdominoplasty)	G&S
Bowel resection	G&S
Breast surgery (lumpectomy)	G&S
Burns debridement	Individual assessment
Cholecystectomy (open)	G&S
Cholecystectomy (laparoscopic)	G&S
Colectomy (formation or closure)	G&S
Ethmoidectomy	Nil
Gastrectomy	2 units
Gastric stapling	G&S
Haemorrhoidectomy	Nil
Hiatus hernia repair (abdominal)	G&S
Hiatus hernia repair (transthoracic)	G&S
Incisional hernia repair	Nil
Laparotomy	G&S
Lipectomy	G&S
Lumbar sympathectomy	G&S
Mastectomy (simple)	G&S
Mastectomy (radical)	G&S
Mastoidectomy	Nil
Pancreatectomy	G&S
Parotidectomy	G&S
Rhinoplasty	G&S
Splenectomy	2 units
Thyroidectomy	G&S
Tonsillectomy	Nil
Tracheostomy	G&S
Vagotomy and drainage	G&S
Varicose veins stripping	Nil

available units in general inventory, another benefit of adhering to recommendations of the MSBOS is that fewer patients with unexpected antibodies will be taken to surgery without appropriate transfusion support. As the MSBOS indicates the extent of pretransfusion testing that should be performed before surgery, adherence to its recommendations will lead to antibody screening being performed on patients with a reasonable chance of requiring intraoperative transfusion, thereby allowing the transfusion service to locate and crossmatch compatible units before the case begins, should unexpected antibodies be detected.

In recipients with red cell alloantibodies who require transfusion, consideration should be given to the time taken to acquire and crossmatch antigen-negative units, and the treating clinical team should be informed. In many cases this is now superseded by use of electronic crossmatching, where greater flexibility and speed are available for issue of blood to patients with blood group records on file and negative current antibody screening results.

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KEY POINTS

1) The transfusion process is unique as it links blood donors with patients in an altruistic, potentially life-saving activity. For many patients there is still no substitute for donated blood components.
2) Prescribers of blood components have a duty of care to their patients to ensure that the benefits of the transfusion outweigh the risks, and a moral obligation to donors to ensure that their donations are used appropriately.
3) The transfusion process is multistep and complex, involving patients and many different staff across the broad spectrum of clinical practice and settings, often working under challenging conditions. In this context there are many opportunities for human error to occur.
4) Investments in quality infrastructure, computerisation and automation and training in the clinical and laboratory aspects of transfusion practice are essential to minimise or, ultimately, prevent errors in the transfusion process.

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Guidelines and Other Resources

For a range of guidelines and other resources on laboratory and clinical hospital transfusion practice:
AABB: http://www.aabb.org/news-resources
Australian and New Zealand Society of Blood Transfusion: www.anzsbt.org.au
British Committee for Standards in Haematology: https://b-s-h.org.uk/guidelines
Canadian resources: www.transfusion.ca, www.transfusionontario.org, https://professionaleducation.blood.ca/en/transfusion
International Society of Blood Transfusion: www.isbtweb.org
Joint Commission on Accreditation of Healthcare Organizations: www.jointcommission.org
Network for the Advancement of Patient Blood Management, Haemostasis and Thromvosis: www.nataonline.com
National Institute for Health and Care Excellence (NICE): https://www.nice.org.uk/guidance/ng24
World Health Organization: https://www.who.int/health-topics/blood-transfusion-safety

26 Disaster Preparedness

Heidi Doughty[1,2], Fateha Chowdhury[1,3] and Richard Rackham[1]

[1] *NHS Blood and Transplant, UK*
[2] *NIHR Surgical Reconstruction and Microbiology Research Centre, Institute of Translational Medicine, Birmingham, UK*
[3] *Imperial College Healthcare NHS Trust, Imperial College, London, UK*

Transfusion support is an essential element of modern healthcare and therefore should be considered in disaster preparedness. In addition, many national civil contingency arrangements require healthcare providers to prove that they can deal with emergencies while ensuring other critical services. Transfusion communities refer to these arrangements as 'blood supply contingency' [1], 'emergency' [2] or 'disaster preparedness' [3]. Preparedness is a dynamic, collaborative process that actively identifies and manages potential and emerging threats. Terrorist events during the 2000s, together with changing trauma and transfusion practice, have stimulated a renewed interest in transfusion emergency preparedness. In addition, disasters such as extreme weather, denial and disruption of computer services and global pandemics also challenge transfusion services. Planning and preparation are essential to protect patients. In this chapter, we consider some of the transfusion principles and practical steps available to prepare for emergencies.

Emergency Preparedness

Disasters and Definitions

A disaster can be described as a sudden, calamitous event that seriously disrupts the functioning of a community and causes human, material and economic or environmental losses that exceed the community's ability to cope using its own resources. Disasters are commonly divided into 'natural' and 'man-made', but may be 'hybrid', i.e. a mixture of both. Natural disasters are extreme weather, wildfires, earthquakes and pandemics. Man-made events include terrorist attacks, industrial and other accidents and cyberattacks. It is the combination of hazards, vulnerability and inability to reduce their impact that results in 'disaster' [4].

Less significant events are more common than disasters. The different definitions used depend on impact; see Table 26.1. We generally refer to 'mass casualty events' (MCE) and 'critical incidents' requiring business continuity (BC) principles. There is no

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Table 26.1 Definitions in emergency planning.

- A **disaster** is a sudden, calamitous event that seriously disrupts the functioning of a community and causes human, material and economic or environmental losses that exceed the community's ability to cope using its own resources
- A **major incident** is an occurrence that presents serious threats to the health of the community, or causes such numbers or types of casualties as to require special measures to be implemented
- A **critical incident** is any localised incident where the level of disruption results in the organisation temporarily or permanently losing its ability to deliver critical services
- A **mass casualty event (MCE)** may be defined as a 'single or simultaneous event(s) where the normal major incident response of one or several health organisations must be augmented by extraordinary measures to maintain an efficient, suitable and sustainable response.'

Figure 26.1 The disaster planning cycle.

consistent categorisation, but all require some cessation of normal activity and implementation of emergency measures. Most definitions recognise that with planning and preparation, the adverse impact may be avoided or at least mitigated. The origins of this approach to emergency planning derive from civil defence, but have developed more systematically since the 1970s. Theoretical models vary, but most emphasise a cyclical approach enabling continuous improvement (Figure 26.1).

Emergency Planning

The overarching framework for emergency planning is well established by humanitarian aid organisations. Planning is essential to deliver a coordinated response to the event, maintain business continuity and guide recovery to 'business as usual' (BAU). Any response should be flexible and scalable to deal with a variety of emergency incidents, including combinations of escalating events [5]. Considerations should include the functional, organisational, hierarchical and geographical aspects of the response. Assessment and preventive measures are based on risk analysis together with access to resources.

Specialised emergency planning for healthcare covers the response to external events such as a patient surge, rather than internal emergencies affecting service delivery, which are covered under BC. However, transfusion-specific plans add value to both. Planning should cover all key products and services, including diagnostics, donation, distribution and direct patient care. Coordinating the effort requires clear command and communication arrangements. Globally, emergencies such as blood shortages are common. Therefore, planning should address seasonal and recurrent events, while providing a generic approach to managing the unexpected [6]. There are examples of transfusion emergency plans covering both blood providers and hospitals [1,3–6].

Organisational Preparation

Organisational preparation aims to ensure an overall coordinated approach together with a good local response. Preparation requires training and rehearsal of the plan. Plans should clarify the command structure, roles, communication and warning systems. The details depend on the shape, size and functions of the organisation. Within a larger institution, senior roles within the emergency team should be clarified, e.g. chief executive, accountable emergency officer, record keeper and incident director. The purpose of any leadership team is to ensure

regular review of response objectives, the route to de-escalation, and delegation of response and recovery activity.

Decision making requires prompt, accurate data. Ideally, this should be digitised. Critical information, such as regional impact and consumable stocks, should be made available using either a situation report (SITREP) or a web-available dashboard. The resources for this are best provided from a properly resourced emergency planning team. Directors and senior staff should engage in regular training and exercises with appropriate scenarios. External standards include ISO 22301, the international standard for business continuity management. All staff should be aware of their role and their own preparation (see Figure 26.2).

Blood Supply and Donors

Blood providers prefer to hold sufficient reserve stock to meet initial demand and then replace the stock after the event. However, many also rely on movement of stock and support from other blood providers. Isolated communities may rely on, or choose to use, alternative models of preparedness based on self-sufficiency using high-readiness donors [7]. The system can be further simplified by prioritising collection of tested low-titre group O whole blood from known emergency donors. Careful management of donors is essential to manage supply and prevent local collection and handling capacity being overwhelmed.

There is often a strong urge to donate after a disaster. A classic example was described by Schmidt in 2002 [8]. He summarised the US experience following the attacks on the World Trade Center and the Pentagon on 11 September 2001. Following these concurrent incidents, 475 000 units of blood were collected, but only 258 units were used. Recent terrorist events in Europe have led to a strong but short-lived response from the public. Proactive communication is needed to both manage the response and encourage future appointments to sustain supply.

Clinical Shortage Plans and Transfusion Triage

Transfusion preparedness to manage demand is best developed in close partnership with clinical stakeholders. Examples

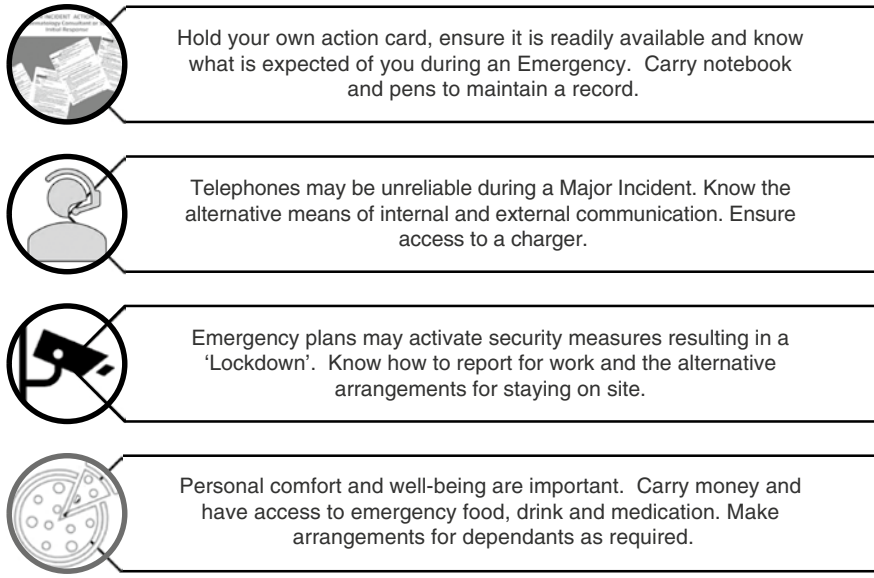

Figure 26.2 Personal preparations.

include shortage plans for components with a limited shelf-life, such as red blood cells [9] and platelets. Such plans may use a traffic light system, where GREEN reflects normal supply; AMBER is when the blood inventory suggests insufficient stock to continue usual transfusion practice; and RED is when the shortage is either severe or prolonged. Such shortage plans are underpinned by practising good patient blood management with principles for prioritisation.

Throughout this chapter we employ the organisational principle of 'transfusion triage'. Triage is the prioritisation of patient care, or victims during a disaster, based on illness/injury, severity, prognosis and resource availability. It is a well-established concept in emergency and prehospital medicine. Triage can similarly be applied throughout the vein-to-vein process of transfusion. For example, the most severely injured patients should be treated first and their blood samples prioritised for testing. Likewise, blood services triage whole blood and apheresis donors to rebuild blood stocks after incidents.

Mass Casualty Events

Interoperability

The response to an MCE should be a multi-agency emergency services response. Many countries have formulated principles for joint working. A good example is the *Joint Emergency Services Interoperability Principles (*JESIP) model used in the UK [10]. The same principles apply to working with other agencies, including the armed forces. Security and safety must come before healthcare, and emergency responders may initially be denied access to a scene until the dynamic risk assessment is complete. Therefore, it is essential that the public are familiar with first aid, especially early and effective haemorrhage control as promoted by the Stop the Bleed® campaign.

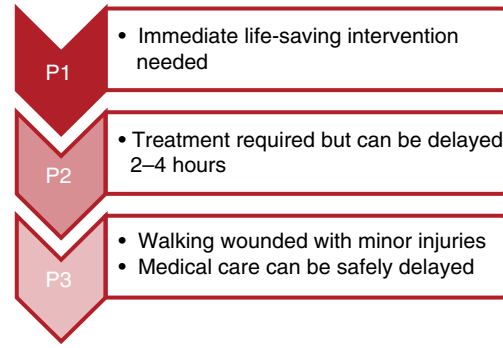

Figure 26.3 A simple prehospital triage system based on priority 1–3.

Once prehospital staff have access, casualties are rapidly triaged to prioritise treatment and evacuation from the scene(s); see Figure 26.3. Priority three (P3) individuals (minor injuries or unharmed) are identified and managed within casualty collection points, often supported by patient welfare teams. The most severely injured (P1) receive life-saving intervention before evacuation to major trauma centres. Those who might need simpler surgery or can wait for treatment (P2) can be treated at smaller hospital units within the trauma network. The transfusion response should be designed to meet a potential surge in demand for blood and distribution of patients across the hospital network.

Major Incident Response

The key to successful management of a major incident is command and control using a common language. A coordination or command team should be stood up to lead and coordinate the response once alerted. Hospitals are usually notified of a major incident by ambulance control, who may contact the switchboard or speak directly to the emergency department. Standard phrases are used to avoid confusion (see Table 26.2). Clear warning enables the hospital teams to discharge or move patients, as well as clearing critical areas and readying them for reception.

'Major incident standby' is used to warn of a potential incident, for example a multiple-vehicle crash, and only a limited number of key staff are alerted together with the blood bank. A full hospital response is started once the incident has been confirmed. It is important that the transfusion team are notified on stand-down. It is worth noting that telephone communications may be unreliable during incidents, as phone lines become overloaded. Alternative means of communication should be considered, including text messaging. The initial information used to determine the response includes:

- Type of incident and injury.
- Anticipated number of patients.
- Estimated time of arrival.
- Anticipated duration of the incident response.

Table 26.2 Standard major incident phrases.

- **'Major incident – stand by':** A major incident is imminent. Warn key staff and assemble the hospital coordination team
- **'Major incident declared – activate plan':** The incident has occurred. A full response is required
- **'Major incident – cancelled':** There is no longer the threat of a major incident
- **'Major incident – stand down':** The last live casualties have left the major incident scene

- Status of the hospital and ability to respond and maintain BAU.

Transfusion Demand Planning

The priority for the hospital transfusion team includes demand planning and organisation of blood stocks and staff. Demand is a product of the estimated number of hospitalised patients and units per patient admitted (UPA), together with a variable demand factor. Past events have seen a predicted demand of two to three times greater than actual blood used. Whereas UPA is traditionally based on use of red blood cells, planning should accommodate changing local practice, including resuscitation based on 'plasma first' or low-titre group O whole blood.

Table 26.3 summarises a recent analysis by Ramsey. He showed that the percentage of patients transfused from an incident varied from 7% to 67% (mean 25%), but that only 5% (range 3–33%) received massive transfusion [11].

The blood use per patient is often less than imagined. Past MCE reviews recommended a UPA of two to four units for hospitalised casualties admitted with bleeding [12,13]. However, there is increasing use of haemostatic components and a difference between the transfusion requirements for general hospitals and specialist trauma centres [11]. Most components are initially ordered as 'universal' components and used within the

Table 26.3 Blood component planning for mass casualty events (MCEs).

Demand = Casualty load × Units per Admission × Demand factors

	Units of blood components per patient admission (UPA)		
Type of facility	RBC (red blood cells)	Platelet dose	Plasma
Trauma centres treating the most severely injured	6	0.5	4
Event-wide medical facilities (also for blood centre planning)	3	0.25	1

This is a summary of proposed blood component planning figures sufficient to cover the balanced transfusion requirements for each patient per admission. The figures are designed to cover 75% of MCEs and are derived from an analysis of incidents using ≥ 50 units of RBCs [11].

first six hours. Substitutions should be accepted where appropriate, e.g. the more plentiful group A plasma used interchangeably with group AB. Some patients may have a continuing demand for blood over days and weeks, especially where repeat surgery is necessary [14]. In addition, the hospital demand for blood may increase once normal and catch-up surgical activity resumes.

Transfusion Risks during Incidents

The biggest transfusion adverse event during emergencies with multiple patients is the accidental transfusion of ABO-incompatible blood due to patient misidentification. The risks may occur during sample collection and labelling, but also at the bedside when initiating the transfusion [15]. Robust laboratory information management system (LIMS)–compatible emergency identification systems are essential to minimise errors. Any change from emergency to routine identification systems requires clear guidance. We recommend that this takes place after the immediate resuscitation and surgical phase. The next biggest risk is delayed transfusions due to a myriad of organisational issues. Both speed and safety are needed in emergency settings. Sometimes it may be safer simply to use a universal donor blood group approach for all. Examples would be group O red cells/whole blood with wider use of RhD-positive blood [16] and group A plasma [17]. Recent events have demonstrated the value of proactively using transfusion coordinators in clinical areas [18]. These staff can assist in a range of activities, including clinical transfusion triage, emergency issue of blood, rapid handling of blood samples and communication.

Transfusion Laboratories

A senior staff member should be appointed as the laboratory lead. Laboratory support includes both sample testing and provision of blood. Laboratory-based testing may be at a premium and near-patient testing (NPT) should be used for non-transfusion testing, where available. Timely ABO and RhD blood grouping is essential if group-specific blood is to be used. Stock management aims to maximise blood for the incident and support other patients requiring urgent transfusion. If the hospital command team choose to cancel non-urgent surgery, the blood no longer required from these areas should be returned for reissue to resuscitation areas. Many hospitals now have remote blood fridges near clinical areas; others may rely on transport boxes. Whichever system is used, transfusion staff should ensure cold chain compliance and traceability. Reordering and movement of stock should be timely. However, it should be noted that road movement may be restricted and delayed by road closures and 'lockdown'.

Business Continuity

MCEs are only one of many events that can challenge transfusion services. Other incidents disrupting supply include adverse weather, power disruptions, cyberattacks and pandemics. The challenge is to balance changes in demand and supply across the complex regulated system that connects donors and patients. In addition, special measures may be required to protect infrastructure in areas at risk of natural disasters or during chemical, biological, radiological and nuclear (CBRN) events [3]. Whereas emergency management seeks to respond to the incident, business continuity provides resilience through focus on the continuity of key business operations and restoration of the organisation to its pre-crisis state.

Concurrency of Events

Disaster responses should consider concurrency and combinations of escalating events. Examples are the seasons which regularly bring multiple challenges for blood services. These include seasonal influenza and other viral diseases, severe weather and their joint effects on a busy health service. In addition, there may be other challenges, particularly

around holiday periods and major events, with the associated influx of visitors and potential shortage of donors. Other less predictable events include major incidents, cyberattacks and pandemics. If two or more of these events occur together, organisations may need to manage simultaneous or interacting events. Planning should be based on a reasonable worst-case scenario, yet still be proportionate.

Loss of Utilities and Information and Communications Technology

BC plans should cover all utilities failures, especially power, equipment and information and communications technology (ICT). Temporary power failure is common, but has a disproportionate impact on modern transfusion services. Critical equipment should be linked to back-up systems or generators. Uninterruptable power supply (UPS) systems can bridge the transition between mains and generator power. In addition, they provide time to safely close down equipment and complete components issuing. Transfusion laboratories are dependent on ICT, not simply for the handling of data, but also for preventing errors. Mitigation should address accessibility to essential transfusion history and safety and the added demands on laboratory staff. Communications are often compromised during disasters and are increasingly dependent on broadband telephony. Alternative resilience options are essential.

Pandemics

The COVID-19 pandemic emphasised the need for global preparedness. During the first wave, blood services experienced staffing pressures and an impact from collecting convalescent plasma leading to a reduction in blood collection [19]. However, this was often mitigated by decreased demand, which also led to unplanned stock build. Other preparations included stockpiling personal protective equipment (PPE), making locations COVID secure, securing new donation venues and eventually vaccination. The steerage of blood collection during these periods required regular cross-functional review from senior leadership teams. The wider planning during the recent pandemic benefited from shared situational awareness, organisational agility and access to modelling systems. An example is shown in Figure 26.4.

Documentation

Major incidents, whether caused by criminal acts or natural causes, may be subject to later investigations. In addition, there is a

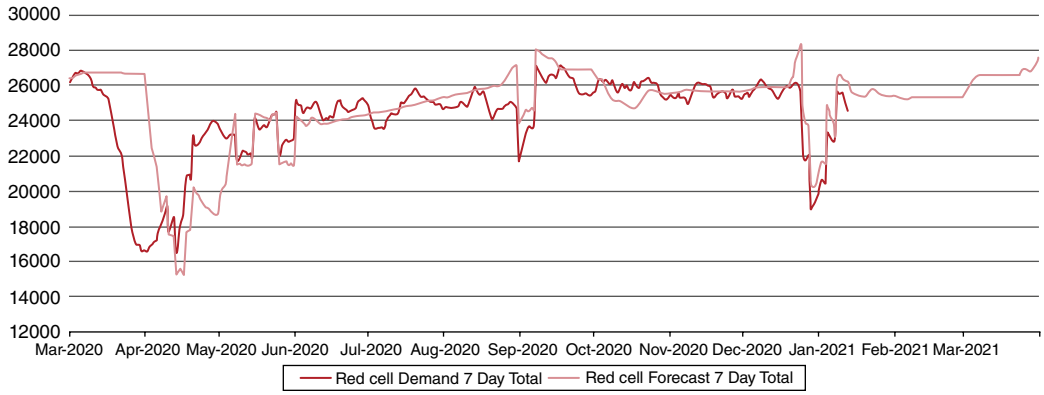

Figure 26.4 An example of the agility of red cell demand planning during the 2020–21 COVID-19 pandemic, showing a seven-day moving red cell demand against forecast. Courtesy of NHS Blood and Transplant, UK.

regulatory requirement for record keeping. Key decisions should be documented and documentation should be accurate and timely. All documentation (electronic and paperwork) must be preserved for collation during recovery. Whiteboards, when used, should be photographed before cleaning. No details should be shared with unauthorised persons.

Recovery Phase

Response and recovery are not discrete activities but should occur simultaneously. The local 'recovery' team should begin to plan recovery activities at the onset of the incident. As soon as the initial response phase is over, the focus should be on returning to BAU, or normality, as soon as possible. A recovery coordinator should lead the overall response, with each department contributing to the effort. The recovery strategy will normally cover some of the key objectives shown in Figure 26.5.

Staff Support and Welfare

Psychologists define resilience as the process of adapting well in the face of adversity. Resilience can be embedded through staff preparation and leadership. However, during the response, the demands on staff may be overwhelming. Practical support should be in place, including food, rest facilities and

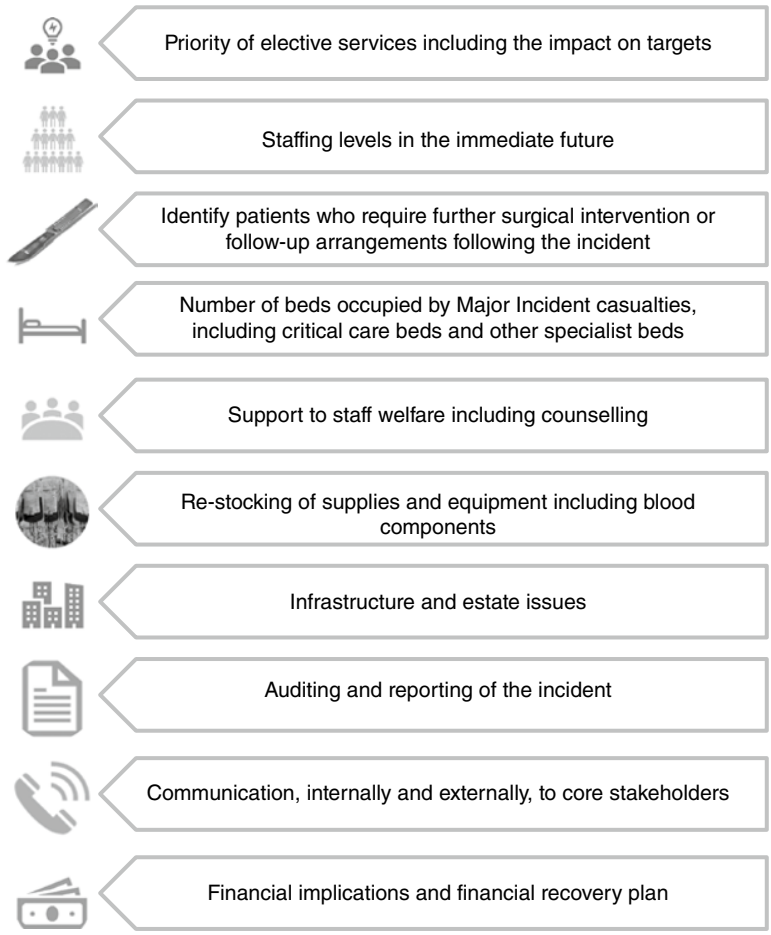

Figure 26.5 Managing the return to normal service delivery.

accommodation for staff unable to travel home [3]. Additional protective measures may be required such as PPE and vaccination. Staff may need sustained practical and psychosocial support following an incident, especially if they, or their friends and family, have been affected [18]. Post-event reviews should support the whole team and sustain the service.

Learning from Incidents

Disasters may be rare or recurrent, depending on where you work in the world. Each event provides an opportunity to learn and improve. Lessons identified should be captured during 'hot debriefs' as soon as practical after the incident. Formal debrief may be held later using additional material. Methodology varies between organisations, but may include one-to-one interviews, questionnaires and responses to 'hot debrief postcards'. Debriefing should be used to thank staff and recognise achievements. The principles of joint organisational learning should then be used across the global transfusion community to enable us all to 'Be Prepared'.

Acknowledgements

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KEY POINTS

1) National emergency planning requires healthcare organisations to prove that they can deal with incidents while maintaining critical services.
2) Transfusion disaster planning should consider concurrency and combinations of escalating events. The response should be flexible and scalable.
3) The transfusion community should be familiar with wider emergency plans and regularly undertake exercises with others to ensure a safe and sufficient supply of blood.
4) Recent mass casualty events and the move to transfusion-based resuscitation in trauma require proactive transfusion support and transfusion triage.
5) The biggest adverse transfusion-associated event in emergencies is ABO error. Robust emergency patient and sample identification systems are essential.
6) Senior staff should lead the response and document key decisions. The response includes preparation for postincident recovery and potential forensic scrutiny.
7) Postincident reviews should support staff, acknowledge achievements, be shared and improve joint organisation learning.

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27 Blood Transfusion in a Global Context

David J. Roberts[1], Imelda Bates[2], Stephen P. Field[3], Aggrey Dhabangi[4], Jean Pierre Allain[5] and Meghan Delaney[6]

[1] *BRC Haematology Theme, Radcliffe Department of Medicine, University of Oxford; NHS Blood and Transplant; Department of Haematology, John Radcliffe Hospital, Oxford, UK*
[2] *Liverpool School of Tropical Medicine, Liverpool, UK*
[3] *Irish Blood Service, National Blood Centre, Dublin, Ireland*
[4] *Child Health and Development Centre, Makerere University College of Health Sciences, Kampala, Uganda*
[5] *NHS Blood and Transplant; Division of Transfusion Medicine, Department of Haematology, University of Cambridge, Cambridge, UK*
[6] *Department of Pathology & Laboratory Medicine Division, National Children's Hospital; Department of Pathology & Pediatrics, The George Washington University, Washington, DC, USA*

> *Of the 118.5 million blood donations collected globally, 40% of these are collected in high-income countries, home to 16% of the world's population.*
>
> (World Health Organization, https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability)

Inequality in the provision of safe blood around the world mirrors the unequal distribution of almost all other resources crucial for effective health services. Unfortunately, in many countries providing safe blood is made more difficult by lack of blood donors and their relatively high frequency of transfusion-transmissible infections (TTIs).

There are several key differences in blood transfusion patterns between wealthy and poorer countries. In the poorest countries, around half of blood transfusions are for children under 5 years of age; whereas in more wealthy countries, patients over 60 years of age account for up to 75% of all transfusions. The majority of transfusions in wealthy countries are for non-emergencies, whereas in poorer countries they are mostly given as emergencies, such as for obstetric haemorrhage, for children with malaria and for trauma. This, combined with increased demands during malaria seasons, makes it especially difficult for blood services to manage supply and demand.

The purpose of this chapter is to inform a wider audience of the problems faced in the development of transfusion services in low- or medium-income countries, predominantly focusing on sub-Saharan Africa.

Safety and Supply

A safe supply of blood is an essential part of medical services and an intrinsic part of any strategy to reduce maternal and childhood mortality. An unstable blood supply is costly in both human and economic terms. Transfusion of infected blood not only causes direct morbidity and mortality, but also

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undermines confidence in the healthcare system. Investment in safe supplies of blood is cost-effective for every country, even those with few resources [1,2]. Blood shortages are very common in poorer countries.

The shockwave of the human immunodeficiency virus (HIV) epidemic put overwhelming emphasis on blood safety, but it is important to maintain focus on ensuring adequate supply of blood. However, balancing the dual goals of adequate supply and minimising infection risks is difficult, especially when resources are severely limited. An adequate and sustainable blood supply is a key component of the healthcare infrastructure and would go a long way to reducing mortality in developing countries, especially among women and children.

In a global modelling study, which used the burden of disease in 180 nations to determine the amount of blood supply needed by diagnosis and compared it to the blood collections reported by each nation, the authors found that 107 countries had insufficient blood stocks to meet their needs [3]. Some nations had up to 75 times greater blood needs than supplies. The countries with the largest relative gap in demand for blood and supply were in sub-Saharan Africa, with only South Africa having enough blood to meet the national need.

The World Health Organization (WHO) has identified four key objectives for blood services to ensure that blood is safe for transfusion:

- Establish a coordinated national blood transfusion service that can provide adequate and timely supplies of safe blood for all patients in need.
- Collect blood only from voluntary non-remunerated blood donors from low-risk populations and use stringent donor selection procedures.
- Screen all blood for transfusion-transmissible infections and have standardised procedures in place for grouping and compatibility testing.
- Reduce unnecessary transfusions through the appropriate clinical use of blood, including the use of patient blood management (PBM) interventions such as alternatives to transfusion, wherever possible.

The WHO also emphasises that effective quality assurance should be in place for all aspects of the transfusion process, from donor recruitment and selection, through to infection screening, blood grouping and blood storage, to administration to patients and clinical monitoring for adverse events.

Testing Blood Products

The WHO recommends that all blood donations should be screened for infections before use. Screening for HIV, hepatitis B (HBV), hepatitis C (HCV) and syphilis should be mandatory. Blood screening should be performed according to quality system requirements.

Screening following basic quality procedures is carried out on 99.8% of the donations in high-income countries and 99.9% in upper-middle-income countries, compared to 82% in lower-middle-income countries and 80.3% in low-income countries. The prevalence of TTIs in blood donations in high-income countries is considerably lower than in low- and middle-income countries.

Local blood transfusion services encounter many problems, including lack of funding, insufficient training, poor management, frequent failure in supply of reagents and consumables and breakdown of the cold chain (mostly related to frequent power cuts). Since 2000, a lot of investment has gone into providing HIV, hepatitis B surface antigen (HBsAg) and to some extent HCV tests in Africa. There have been enormous efforts to ensure that blood collected in Africa is tested for HIV. The residual risk of HBV infection

remains substantial because of donations containing undetected low levels of HBsAg or occult HBV DNA. Recent estimates of the residual risk of transmission in the pre-seroconversion window period are HIV 1 : 3600, HCV 1 : 210 and HBV 1 : 410 in Burkina Faso [4], and HIV 1 : 16 000, HCV 1 : 5000 and HBV 1 : 2000 in Gabon [5]. The wide range of residual risks may reflect the variable local epidemiology, the diverse donor populations and different testing methodologies.

In low-income nations, testing donated blood is limited in terms of both the scope of TTIs screened and the test platforms employed. A survey of blood collection services across India showed that 87% of the donor units were screened for HBV, 95% for HIV, 94% for syphilis, 67% for malaria and 6% for HCV. Only 13% of blood banks used enzyme-linked immunosorbent assay (ELISA) kits for HBsAg. Notification of the occurrence of transfusion-associated hepatitis was provided less than 40% of the time and probably reflects weak haemovigilance systems. Test sensitivity is critical in the face of high prevalence rates for HIV, HBV and HCV in blood donations (Table 27.1 and Box 27.1) [6–9]. Test specificity is critical, because high immunoglobulin (Ig)G levels are reflected in a high frequency of false positives and requires confirmation with alternative affordable assays, including rapid tests. Nucleic acid testing (NAT) is highly effective and has been introduced in South Africa and a few centres elsewhere [17]. However, widespread use of NAT is unaffordable for most countries; cheaper, simpler methods to perform NAT would be useful.

Blood Donors

Recruiting voluntary donors from the community is complex, expensive and depends on regular education programmes, collection teams, vehicles and cold storage. It is very difficult to expand the number of volunteer donors in poorer countries [18]. This challenge is illustrated by the finding that several nations in sub-Saharan Africa have recently achieved 100% voluntary non-remunerated blood donations (VNRBDs), but still fall below the WHO recommended 10 donations per year for each 1000 of the population.

Some potential donors are fearful of HIV testing or distrustful of the health system. There are also cultural beliefs surrounding blood donation that inhibit donors from coming forward. Some of these appear to be misinformation about donating blood (e.g. ‘men will become impotent if they donate

Table 27.1 Prevalence of transfusion-transmissible infections in blood donations – median (interquartile range, IQR) – by income groups.

	HIV	HBV	HCV	Syphilis
High-income countries	0.001% (0–0.01%)	0.01% (0.003–0.13%)	0.06% (0.002–0.05%)	0.01% (0.002–0.11%)
Upper-middle-income countries	0.10% (0.03–0.23%)	0.29% (0.15–0.62%)	0.18% (0.06–0.35%)	0.34% (0.11–1.08%)
Lower-middle-income countries	0.19% (0.03–0.77%)	1.96% (0.76–5.54%)	0.38% (0.03–0.80%)	0.69% (0.16–1.25%)
Low-income countries	0.70% (0.33–1.66%)	2.81% (2.00–4.50%)	1.00% (0.50–2.23%)	0.92% (0.60–1.81%)

HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

Box 27.1 Epidemiology of blood-borne infections in sub-Saharan Africa.

Human immunodeficiency virus (HIV)

The overall prevalence of HIV antibody in sub-Saharan Africa by region ranges between 0.5% and 4%, although some urban, adult populations have prevalence rates of 10–15% [10]. In donors, it tends to be below 5% in West Africa, below 10% in East and Central Africa and above 10% in southern Africa [6–9].

Hepatitis B (HBV)

Chronic hepatitis B prevalence, indicated by the presence of circulating hepatitis B surface antigen (HBsAg), ranges between 5% and 25% of the population including blood donors [11]. HBsAg is more prevalent in West Africa (10–25%) than in East or Central Africa (5–10%); the lowest prevalence is found in southern Africa (5% or less), in part related to HBV genotypes. These high prevalence rates are due to (vertical) transmission at birth or (horizontal) infection in infancy. It is envisaged that following the roll-out of national vaccination programmes of infants at 6 weeks, initiated in many sub-Saharan African nations in the 2000s, will substantially reduce the burden of HBV in blood donations in the decade ahead.

Hepatitis C (HCV)

Antibody to HCV is not uniformly screened for in many parts of Africa, but the prevalence of this infection ranges between 0.5% and 3% and reaches 10–15% in Egypt [12]. The prevalence may be high locally, suggesting the importance of specific local factors that are poorly understood.

Other Infections

Most countries in sub-Saharan Africa do not screen for human T-lymphotropic virus (HTLV), since the prevalence is low (< 2%). Although the risk of acquiring syphilis from infected blood is low, most blood banks in sub-Saharan Africa do screen for *Treponema pallidum* with poorly specific assays. Fresh blood is potentially infectious for syphilis, but storage at 4 °C for over a week can inactivate the bacterium.

Malaria is transmitted by transfusion even in semi-immune recipients. In areas of low or no malaria transmission, screening for the parasite or excluding antiplasmodium-positive donors is important, as recipients are likely to have no immunity. In countries where malaria is highly endemic, the prevalence of *Plasmodium* in donor blood is often very high (16–55%) [13]; excluding donors with low-grade parasitaemia is often impracticable and so malaria chemoprophylaxis among the most vulnerable patient populations receiving transfusion appears to be a more practical strategy, as recommended by the World Health Organization [14].

Bacterial contamination of blood components is under-recognised and may reach 10% of products at the time of issue [15,16].

blood'; 'HIV can be caught from the blood bag needle'). There are, however, other cultural beliefs related to understanding the value of blood to the individual and to society, for example that blood is related to kinship. This partly explains why donors in low- and middle-income countries may prefer to donate for people and communities they know rather than into a national or regional supply system. Understanding local beliefs surrounding blood and blood donation is important in developing effective donor recruitment and retention strategies [19]. It is worth noting that similar problems were a barrier to widespread acceptance of blood donation in London over 75 years ago.

As volunteer donors are in short supply, family members are frequently asked to provide blood for their relatives in hospital. This

is called replacement donation and many of these donors perceive themselves to be voluntary donors [20]. In 2002, in Africa as a whole, the WHO estimated that over 60% of blood originated from replacement/family donors. In sub-Saharan Africa, the proportion of blood derived from replacement donors is likely to be higher. The aim should be to maximise conversion of voluntary and replacement donors into regular donors, since those who are successful repeat donors have the best safety profile.

Use of Blood Products

Many clinical guidelines, albeit based on consensus opinion rather than well-defined evidence, suggest that transfusions for children are indicated if haemoglobin (Hb) < 40 or 50 g/L with symptoms of decompensation [1,21]. A recent study randomly allocated 3196 severely anaemic African children to receive either 30 mL/kg or 20 mL/kg of whole blood and found that there was no significant difference in deaths at 28 days. However, there was significant heterogeneity in the findings, since among the 1253 children (39.2%) with fever, mortality was higher with 30 mL/kg transfusion than with 20 mL/kg [22].

These findings have prompted an International Consensus Conference to develop guidelines for the management of children with severe anaemia in Africa [23]. The key findings of the trial and the consensus conference are summarised in Box 27.2 and represent a significant advance in understanding effective and safe clinical practice for the management of severe anaemia in children in Africa. These studies will prompt much further research to understand the basis of these findings and their wider application.

Pregnant women are the second most common recipients of blood, particularly for haemorrhagic emergencies.

The WOMAN trial showed that prompt tranexamic acid reduces death due to bleeding in women with postpartum haemorrhage, with no adverse effects [24]. It should be given no more than three hours after haemorrhage (see WHO guideline 2017 in Further Reading).

Significant quantities of blood are also used in trauma, often related to motor vehicle accidents, and in surgery and general medicine. There is now good evidence from the CRASH-2 trial conducted in 40 countries around the world that tranexamic acid (loading dose 1 g over 10 min, then infusion of 1 g over 8 h) reduces death [25]. Early treatment within three hours of injury is essential (see CRASH-2 Collaborators in Further Reading). Beyond these pivotal trials, there are few systematic reviews and international guidelines covering the use of blood and blood products, and the scope for improving clinical practice and reducing unnecessary transfusion is substantial.

The problems surrounding the rapid supply of safe blood have prompted the alternative use of autologous blood transfusion through a cell saver in some settings for elective procedures. There are logistical and training problems to be overcome; however, this appears to be a potential route to decrease the barrier of elective transfusion in certain settings.

As low- and middle-income countries improve their public and primary healthcare, chronic medical illnesses, such as cardiac disease, diabetes and cancer, become more of a priority for healthcare services. To be able to provide therapies for these disorders, especially for cancer, transfusions are needed to support patients with chemotherapy-induced bone marrow suppression. Unfortunately, in some instances the unreliability of blood supply to support elective blood transfusion may force treating physicians to choose less rigorous chemotherapeutic regimens, which can affect long-term outcomes. Initiatives to improve cancer care and cardiac surgery in low- and middle-income countries must also include attention to blood and component supply and safety to support patients through intensive treatments.

Box 27.2 Transfusion management of severe anaemia in African children [23].

The phase III Transfusion and Treatment of severe anaemia in African Children Trial (TRACT) showed the following:

- Conservative management of uncomplicated severe anaemia (haemoglobin [Hb] 40–60 g/L) was safe.
- Transfusion volume (20 vs 30 mL/kg whole blood equivalent) for children with severe anaemia (Hb < 60 g/L) had strong but opposing effects on mortality, depending on fever status (> 37.5 °C).

In 2020 a stakeholder meeting of paediatric and blood transfusion groups from Africa reviewed the results and additional analyses and concluded:

- The definition of severe anaemia can be standardised as Hb < 60 g/L.
- Children with uncomplicated severe anaemia do not require immediate transfusion, but do require monitoring, because ~50% will develop severe and life-threatening anaemia requiring subsequent transfusion.
- Clinical and Hb monitoring reviews are needed in a child with severe anaemia (both uncomplicated and complicated) to identify those needing transfusion.
- The most effective and safest volume of blood for transfusion depends on the axillary temperature when a blood transfusion is ordered:
  - If temperature is ≤ 37.5 °C, then transfuse 30 mL/kg whole blood or 15 mL/kg red cell concentrate.
  - If temperature is > 37.5 °C, then transfuse 20 mL/kg whole blood or 10 mL/kg red cell concentrate.
- There is no need for separate recommendations for children with malaria (even those with high parasite burdens).
- Optimal transfusion of sickle cell disease (SCD) patients or of children with poor nutritional status may need further specific studies.
- A high proportion of children presenting with severe anaemia had undiagnosed SCD. Admission to hospital with severe anaemia should prompt testing for SCD for long-term follow-up and infection prophylaxis.

Systems

Taken together, the low availability of blood donors, cold chain and pathogen testing leads to low stocks of blood and blood products, which ultimately affects patient care and outcomes. Patients in low- and middle-income countries may present late in the course of their disease, and the need for urgent transfusion coupled with shortages of blood mean that patients may die before a blood transfusion can be organised. In situations where blood must be donated by relatives before a patient can be transfused, by the time a donor has been found, screened and venesected, and the blood is transfused into the patient, several hours or even days can elapse. The process can be speeded up if relatives are asked to donate after the patient has been transfused with blood from the hospital's stocks. In this way, a combination of voluntary donations to maintain some emergency stocks with posttransfusion donations from patients' relatives may provide a practical solution to blood shortages or a means to keep a blood bank with adequate inventory.

It is axiomatic that transfusion medicine should be incorporated into national health plans. The WHO has provided a recommended structure of national blood transfusion services. It suggests that at the national level, the transfusion service should have a medical director, an advisory committee and national transfusion policies and strategies, with the appropriate statutory instruments to ensure the national coordination and standardisation of blood testing, processing

and distribution (see Further Reading). Notwithstanding these recommendations, transfusion activities must be integrated with other services at the community level.

There has been progress towards realising the WHO's recommendations for national blood programmes. In 2016, the WHO estimated that among the 46 member states in the African continent, 38 had a national blood policy and 19 had a policy to specifically encourage and develop a system of VNRBD.

It is worth reflecting on why the development of national transfusion services has not been achieved. A key reason is that it is expensive and logistically complex. Management skills to run such services are insufficient and the cost is high for central health care ministries to bear.

When a transfusion service is provided by individual hospitals, it places an enormous burden on laboratory resources. One survey showed that in a typical district hospital in southern Africa, the overall cost of the transfusion service, including consumables, proportional amounts for capital equipment, staff time and overheads, was 36% of total laboratory costs. In hospital-based systems that depend on replacement donations, it is the patient's family that bear the cost of donor recruitment.

Although there are benefits in having a national blood service (such as purchasing power, standardised testing and quality assurance), some of these activities may incur additional costs (e.g. quality assurance, local education programmes, dedicated collection team(s), vehicles and cold storage). In addition, a national service must solve the very real practical problems of stock tracking and maintaining regular distributions of blood to remote facilities. A unit of blood in a centralised service costs around four times as much as one from a hospital-based system that uses family replacement donors; this does not include capital costs (which range between $25 and $150). Precise cost–benefit analyses for the use of blood have not been carried out, making nationalised planning even more difficult.

A perennial problem in healthcare systems is the availability of skilled technical staff; this may be compounded by internal migration of technical staff from hospitals to national or regional centres or the private sector. There is a severe lack of training and career advancement opportunities for technical and clinical blood service staff. Training programmes to increase capacity for the processing, testing and issue of blood, and also for research into how to improve the effectiveness of blood services in poorer countries, are therefore an integral part of service development.

Improvements

Putting the World Health Organization Objectives into Practice in Sub-Saharan Africa

Blood is an expensive commodity in relation to the annual per capita budget for healthcare in low- and middle-income countries and there is little evidence that centralised blood services, which in such countries are predominantly externally funded, can reach an adequate blood supply and be sustained. However, a concerted effort was made to develop an integrated transfusion service in Nigeria (Box 27.3), and the progress and problems of developing a national service delivering a safe and sufficient blood supply have been reviewed by Aneke and Okocha (see Further Reading). Whatever budget is available, the problems surrounding the supply, safety, cost and use of blood must be addressed. There must be a balance between providing an ideal, integrated national service and the more pragmatic solutions afforded by local services.

Improving the Blood Supply

Careful donor selection is crucial, not only to improve the supply of blood but also to

Box 27.3 Towards development of a national transfusion service in Nigeria.

In 2004, Nigeria, the most populous country in Africa, had a highly fragmented hospital-based transfusion system. There was little coordination from central government and most of the blood came from replacement and paid donors. Testing for transmissible disease markers was inconsistent. Family replacement donors in a hospital-based blood service were the most economical option, but the blood supply was insufficient in face of high maternal and childhood mortality. There was therefore a need to change practice.

The Safe Blood for Africa Foundation, with a grant from the United States Agency for International Development (USAID) and later the President's Emergency Plan for AIDS Relief (PEPFAR), established a demonstration blood service in the capital, Abuja. This service:

- Collected its blood from voluntary unremunerated donors in the local community.
- Tested donor samples for human immunodeficiency virus (HIV), hepatitis B and C and syphilis.
- Distributed blood to the local hospitals.
- Established a quality management system with standard operating procedures (SOPs) written and followed.

The Federal Ministry of Health has established six zonal transfusion centres under the umbrella of the national blood transfusion service and overseen development of national blood policy and national guidelines for standards of transfusion practice; 10 states have opened transfusion centres.

The Safe Blood for Africa Foundation provided technical assistance for the establishment of these centres, training staff in all elements of transfusion and helped establish nucleic acid testing (NAT) in some centres.

The major problem was recruiting blood donors. Young people were encouraged to donate with the establishment of a Club 25 programme. The problems encountered include low uptake of voluntary non-renumerated donation, high donor deferral rates due to low haemoglobin, high rates of donors screening positive for potential transfusion-transmitted infections, and limited implementation of patient blood management interventions to conserve blood and increase appropriate use of blood.

reduce TTI risk. The selection of volunteer donors from lower-risk populations is considered the most effective approach, and considerable effort has been devoted to promoting voluntary, repeat donations. In practice, these are often secondary school students, with median age ranging between 16 and 20 years. They are younger and have a greater proportion of females than family donors, but there are some concerns that although these younger donors have a lower prevalence of TTIs than older donors, they may have a higher incidence of new infections. Experience has shown that while recruiting volunteer donors in schools can be relatively inexpensive, encouraging them to become repeat donors is difficult and expensive. Blood services are now focusing more efforts into motivating both volunteer non-remunerated and family replacement donors to donate blood repeatedly to provide safer blood [26,27].

Several strategies have been devised to encourage repeat donors and thus reduce the risk of virus carriage. In Zimbabwe, Pledge 25 Club, a programme using education and incentives to attract school students to give blood 25 times, has been successful. Similar, less ambitious schemes, for example a 'Club 5', could also be effective. The WHO slogan 'Safe blood starts with me' has also resulted in educational programmes around the world. These schemes can be complemented by strategies

to recruit donors from faith-based organisations or collaborating with radio stations to organise and promote blood donations. Specific strategies intending to encourage family replacement donors to become repeat donors are being developed.

Improving Screening for Blood-Transmitted Infections

New approaches to blood donor testing for TTIs have been adapted to local situations and appear promising. In small blood banks, the expensive micro-titre plate systems used post donation can be replaced by cheaper, more cost-effective, high-performance rapid tests such as the fourth-generation HIV point-of-care tests performed pre or post donation. Predonation testing provides the advantages of reducing material waste, with on-site communication with deferred donors who could not otherwise be reached [28]. Rapid immunochemical and nucleic acid dipsticks are being developed for blood-borne pathogens and may cut the cost of pre- and postdonation testing to a tenth of present costs. The WHO has established systematic evaluations of both ELISA and rapid tests to guide developing countries in their choice of tests. These evaluations include test costs. Many rapid tests for anti-HIV and HBsAg and fewer for anti-HCV are available, but sensitivity and specificity, ease of use and cost vary greatly. Some of these tests are performed in a single step, with results obtained in 10–20 minutes using whole blood, plasma or serum samples. The best assays have sensitivity similar to ELISA for anti-HIV, detect 0.2 ng/mL of HBsAg and have > 99% sensitivity for anti-HCV and > 99% specificity.

Blood safety has often focused on the risk of viral infection in donors, but bacterial contamination of units is also a substantial problem. Two studies have highlighted the considerable risk of bacterial infection in nearly 10% of whole blood units [12,13]. Contamination appears to be of environmental rather than of donor origin, and reducing these hazards will be an important challenge in the future. Pathogen-reduction methods applied to whole blood and compatible with component preparation might present substantial advantages if affordable [29].

Further, interventions aimed at minimising transfusion with ABO-incompatible blood components such as positive patient identification, as well as haemovigilance systems, need development. They represent complementary strategies for improving transfusion safety in sub-Saharan Africa and other low-income settings.

Meeting the Financial Requirements of Transfusion Services

The challenge for poorer countries is that enough safe blood should be available for health services and individuals, even when resources are extremely limited. The high cost of providing blood makes it impossible to recoup the cost of blood by user fees alone and blood services will require internal or external public funding for the foreseeable future [30]. Developing systems that rely more on local resources means that in the long term they may be more flexible, productive and sustainable.

Improving the Clinical Use of Blood: Guidelines for Transfusion Practice

The use of guidelines can reduce unnecessary transfusions and many institutions in sub-Saharan Africa and Asia have developed guidelines to promote the rational use of blood and blood components (see Further Reading). The scope for improvement in clinical practice is great. For example, strict enforcement of a transfusion protocol in a Malawian hospital reduced the number of transfusions by 75% without any adverse effect on mortality. However, the difficulties of changing behaviours in the long term and outside the context of a research study are not to be underestimated, and such changes in clinical practice may take place over 5–10 years, like the progressive implementation of patient blood management in higher-income countries (see Chapter 37).

The principles underlying most transfusion guidelines are similar and combine a clinical assessment of whether the patient is developing complications of inadequate oxygenation with measurement of their haemoglobin (as a marker of intracellular oxygen concentration). In the USA, anaesthetists suggest that transfusions are almost always indicated when the Hb concentration is less than 60 g/L, whereas in many sub-Saharan African countries transfusions are recommended for children at Hb concentrations less than 40 g/L, provided there are no other clinical complications. The TRACT trial has now obtained good evidence for conservative management of uncomplicated severe anaemia (Hb 40–60 g/L) and for modulating the transfusion volume depending on fever status (see above) [22,23].

Moreover, the lack of fractionated blood products and the reliance on whole blood should be considered in context. Using whole blood for many of the common emergency indications for transfusion in Africa may be advantageous, as it supplies critical coagulation factors for patients facing haemostatic challenge, such as in the setting of postpartum haemorrhage and following significant trauma. It is thought that packed red cells may be best for patients with a delicate fluid balance and significant anaemia. However, a recent review of the published literature showed that there is virtually no published evidence for or against the use of whole blood versus packed red cells for obstetric bleeding or anaemia, or paediatric anaemia in sub-Saharan Africa [31].

Pandemics

The outbreak of Ebola virus in three West African countries killed many thousands of people. At the time of the outbreak, there was no proven therapy for the disease; however, 40–65% of patients made a full recovery. Trials of convalescent plasma containing antibodies against the virus were not effective in improving outcomes. To date, convalescent plasma has not been shown to be effective against the SARS-CoV-2 virus in hospitalised patients, however, further trials of plasma with high-titre antibodies from vaccinated individuals have shown efficacy when given early, epidemics have highlighted the needed for well-organised hospital services to coordinate preventive measures and deliver acute clinical care. It remains possible that blood centres could play a significant role in providing treatment if trials of convalescent patients show efficacy in the early treatment of vulnerable and/or immunocompromised patients.

Conclusion: The Future of Blood Transfusion in a Global Context

Fulfilling the first WHO objective of establishing a coordinated national blood transfusion service that can provide adequate and timely supplies of safe blood for all patients in need has proved to be very difficult in many countries, even given substantial external funding. Nevertheless, some countries have made progress and over the last two to three decades have established national transfusion services. On the other hand, progress has been made by developing local services, and there must be a balance between providing an ideal, integrated national service and the more pragmatic solutions afforded by less costly, local services. There remains considerable scope to optimise fluid management and other ancillary treatments, and patient blood management interventions to reduce unnecessary transfusions through blood conservation and appropriate clinical use of blood and products.

Increased blood supply depends on the recruitment of all types of non-remunerated

donors, whether volunteer non-remunerated donors or family replacement donors, and the development of innovative strategies to encourage both groups of donors to give blood regularly.

Resources must be made available by governments to ensure that the essential supplies are available, such as blood bags, grouping reagents and test kits, and laboratory and blood bank management systems (ideally electronic) also need to be improved to ensure effective testing and processing and the maintenance of the cold chain. Hospitals and other health facilities could cooperate to directly purchase cheap, high-quality tests adapted to their needs. Significant efforts need to be made to ensure that blood services in poorer countries are underpinned by feasible and sustainable internal financing mechanisms so they can operate independently.

There is currently a feeling of guarded optimism about the future of blood supply and safety in developing countries. The allocation of resources for the prevention of HIV across the world, including the investment by governments of wealthy countries and contributions from international and private agencies, indicates the importance of reducing HIV transmission through blood, but runs the risk of neglecting other basic laboratory services, such as blood grouping and haemoglobin measurements. Parallel to the price reduction for antiviral drugs, the cost of screening tests supplied to developing countries has also decreased. The high cost of anti-HCV testing should now be reduced, as the patent has expired in Europe. More effective and efficient methods for testing blood are to be welcomed and pathogen-reduction methods applicable to whole blood would be an enormous relief, if affordable. The real challenge will be to integrate improvements in the supply and safety of blood in sustainable, coordinated transfusion services with strong community engagement.

KEY POINTS

1) In the last two to three decades, nearly all African states have created a national blood policy, but only just over half have been able to implement their policies.
2) The main obstacles to implementation are a lack of trained staff, the high cost of blood and testing in relation to healthcare budgets, and recruitment of donors.
3) In the absence of centralised services, facilities rely on blood collected by hospitals from family or replacement donors.
4) The high rate of chronic viral infections in populations implies that the residual risk of infection of human immunodeficiency virus (HIV) and hepatitis B infection remains substantial with enzyme-linked immunosorbent assay (ELISA) testing.
5) Several initiatives are being trialled to improve the supply and/or safety of blood by encouraging repeat voluntary donors, reviewing donor testing strategies, developing systems that rely more on local resources, pathogen-reducing whole blood, researching methods for low-cost nucleic acid testing, and improving clinical practice through guidelines and audits of the use of blood.

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28 Inherited and Acquired Disorders of Haemostasis

Irina Chibisov, Jansen Seheult and Franklin Bontempo

University of Pittsburgh Medical Center; Vitalant, Pittsburgh, PA, USA

Normal Haemostasis

Haemostasis is an interconnected series of events involving the interaction of the vascular endothelium and subendothelial matrix, platelets, procoagulant enzymes and cofactors, natural anticoagulants and fibrinolytic enzymes, which maintain the integrity of the circulatory system. In a normal individual, there is a balance between procoagulant and anticoagulant activities and any disruption may lead to bleeding or clotting disorders.

The generation of thrombin is the key to successful haemostasis. Historically, a 'cascade' model developed in the mid-1960s described the intrinsic and extrinsic pathways where inactive clotting factors were converted in a cascading fashion to activated enzymes, ultimately resulting in the generation of thrombin and the conversion of fibrinogen to fibrin (Figure 28.1). Although this model provided an adequate explanation of plasmatic haemostasis observed in laboratory coagulation assays, it was inadequate to explain many clinical problems. The 'cell-based model' provides a more comprehensive approach to understanding the *in vivo* coagulation process [1]. In this model, coagulation takes place in three overlapping phases on cellular surfaces within the vasculature: *initiation, amplification* and *propagation* (Figure 28.2).

To prevent excessive, inadequate thrombosis, there are built-in mechanisms to control the procoagulant response. These include tissue factor pathway inhibitor (TFPI), which inactivates the TF–FVIIa (factor VIIa) complex; antithrombin, which complexes with and inactivates FIXa, FXa, FXIa and thrombin; and the thrombomodulin, protein C and protein S pathway, which inactivates FVa and FVIIIa. Fibrinolysis is also part of the normal haemostatic response. Plasminogen is incorporated into the forming clot, activated to plasmin by proteases, such as tissue-type plasminogen activator released from the damaged or activated endothelium. Plasmin cleaves cross-linked fibrin to form D-dimers and other fibrin degradation products (FDPs), and contributes to tissue remodelling.

Investigation of Abnormal Haemostasis

Abnormalities in the haemostatic system may be congenital or acquired. Clinical presentation can vary from mild to life-threatening haemorrhage. The most important element in the evaluation of patients with haemostatic abnormalities is a careful personal and family history, their symptoms and clinical signs, including type of bleeding, bleeding following dental work, surgery or childbirth, and objective evidence of bleeding such as development

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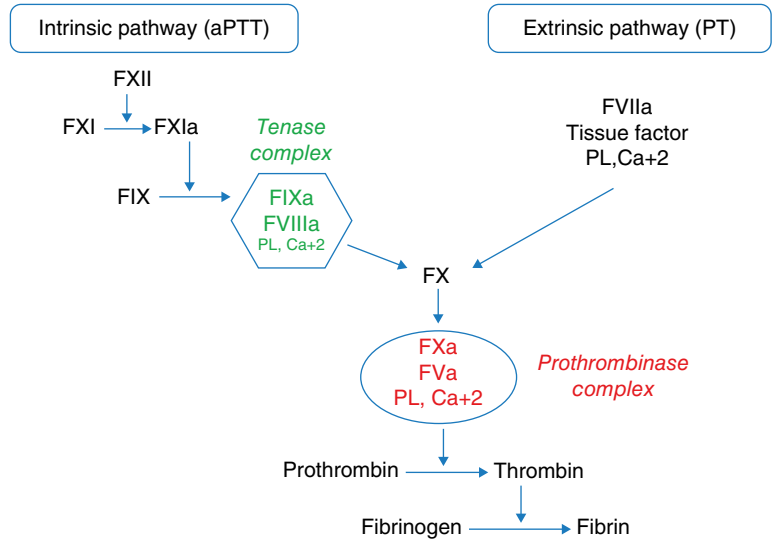

Figure 28.1 'Cascade' model of coagulation.

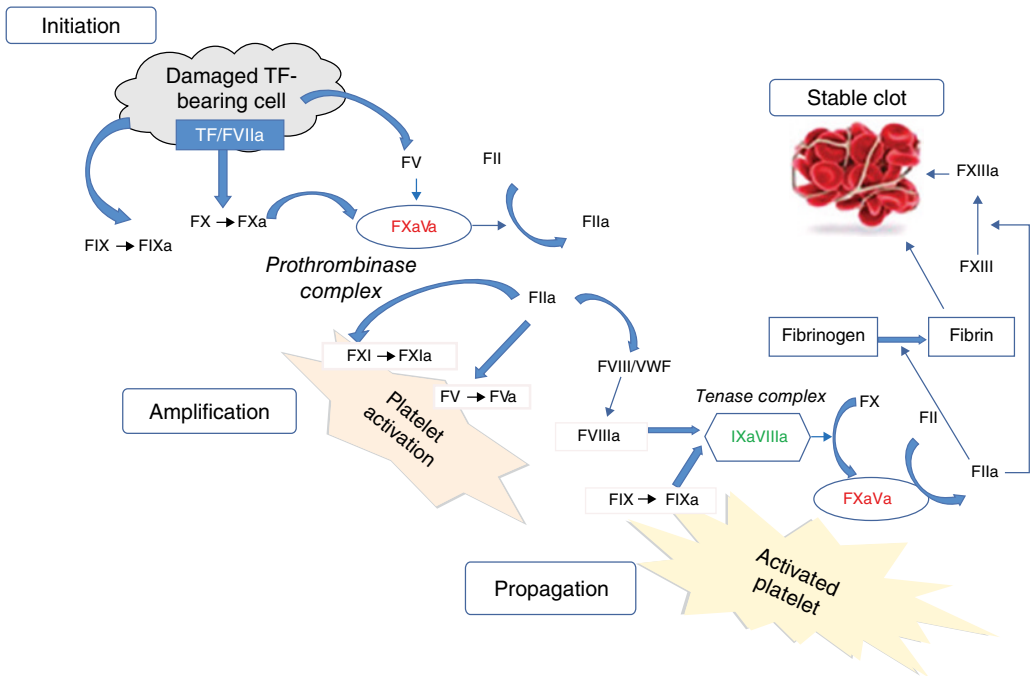

Figure 28.2 Cell-based model of coagulation. Coagulation is initiated on the surface of tissue factor (TF)-bearing cells, generating small amounts of thrombin (FIIa). In the amplification phase, platelets and factors V, VIII and XI are activated. In the propagation phase, large amounts of thrombin are generated, leading to activation of fibrin and factor XIII and production of cross-linked stable clot.

of anaemia, requirement for transfusion or surgical intervention. However, history may be unavailable in certain settings, such as in trauma resuscitation, and decisions must instead be guided by clinical judgement, supplemented by laboratory assays.

The initial laboratory investigation should include a full blood count with platelet

count, peripheral blood film and screening tests of haemostasis, including the prothrombin time (PT) and activated partial thromboplastin time (aPTT). Additional screening tests that can be performed are listed in Table 28.1. Point-of-care testing or near-patient testing can also provide invaluable information, especially in the emergent setting. For example, whole blood viscoelastic haemostatic assays (VHA), including thromboelastogram (TEG®) and rotational thromboelastometry (ROTEM®), provide an almost global evaluation of haemostasis (Table 28.2 and Figure 28.3).

Inherited Haemostatic Defects

Haemophilia A

Congenital haemophilia A is an X-linked recessive disorder of reduced or absent factor VIII activity that accounts for 80–85% of all cases of congenital haemophilia. Although most patients have a family history, up to 30% of cases are associated with sporadic mutations. Acquired haemophilia A is extremely rare, typically affects older adults and is due to the formation of an autoantibody against factor VIII.

Table 28.1 Laboratory haemostasis screening tests.

System	Test	Abnormality
Coagulation	↑PT, normal aPTT*	Factor VII
	↑aPTT, normal PT*	Factors VIII (including vWD/AvWS), IX, XI, XII, prekallikrein, high molecular weight kininogens
	↑PT, ↑aPTT	Factors II, V, X
	↑TT	Hypo- or dysfibrinogenaemia Heparin and direct thrombin inhibitors
	Fibrinogen (Clauss assay)	Hypo- or dysfibrinogenaemia
Platelets	Platelet count and peripheral blood film inspection	Thrombocytopenia
	Platelet function (PFA-100™ or PFA-200™)	Acquired or inherited disorders in platelet function, vWD/AvWS, antiplatelet drugs, herbal supplements, dietary compounds, thrombocytopenia, low haematocrit
	Light transmission platelet aggregometry	Acquired or inherited disorders in platelet function, vWD/AvWS (ristocetin), antiplatelet drugs, herbal supplements, dietary compounds
	VerifyNow™	Antiplatelet drugs (aspirin, P2Y12 inhibitors)
Fibrinolysis	D-dimers	DIC, liver disease, TIC, thrombosis
	Fibrin degradation products	DIC, liver disease, TIC, thrombosis, primary hyperfibrinolysis
	Euglobulin clot lysis time	↓Factor XIII deficiency, thrombolytic therapy, DIC, trauma, liver disease, cardiopulmonary bypass ↑DIC, trauma, liver disease/transplantation
VHA	TEG/ROTEM	Evaluation of factor deficiency, anticoagulation, hypo- or dysfibrinogenaemia, thrombocytopenia/platelet dysfunction, fibrinolysis

*PT and aPTT reagents have variable sensitivities to common pathway factors (II, V and X) and therefore common pathway factor deficiencies may also result in these screening test abnormalities, depending on the reagents used in the laboratory.

aPTT, activated partial thromboplastin time; AvWS, acquired von Willebrand's syndrome; DIC, disseminated intravascular coagulation; PT, prothrombin time; ROTEM, rotational thromboelastometry; TEG, thromboelastography; TIC, trauma-induced coagulopathy; TT, thrombin time; VHA, viscoelastic haemostatic assay; vWD: von Willebrand disease.

Table 28.2 TEG/ROTEM parameters, selected abnormalities and treatment.

TEG Parameter	ROTEM Parameter	Situations in which they are changed	Treatment
R	Clotting time (CT)	Prolonged in coagulation factor deficiency, anticoagulation	Plasma
K	Clot formation time (CFT)	Prolonged in coagulation factor deficiency, fibrinogen deficiency, platelet dysfunction, thrombocytopenia	Based on combination of other parameters
α angle	α angle	Decreased in hypo- or dysfibrinogenaemia (15–20% contribution from platelets)	Cryoprecipitate
Maximal amplitude (MA)	Maximum clot firmness (MCF)	Decreased in thrombocytopenia, platelet dysfunction (15–20% contribution from fibrinogen)	Platelets, DDAVP®
Lysis index at 30 min post MA (LY30)	Lysis index at 30 min post MA (LI30)	Increased in excessive fibrinolysis	Anti-fibrinolytics (tranexamic or aminocaproic acid)

ROTEM, rotational thromboelastometry; TEG, thromboelastography.

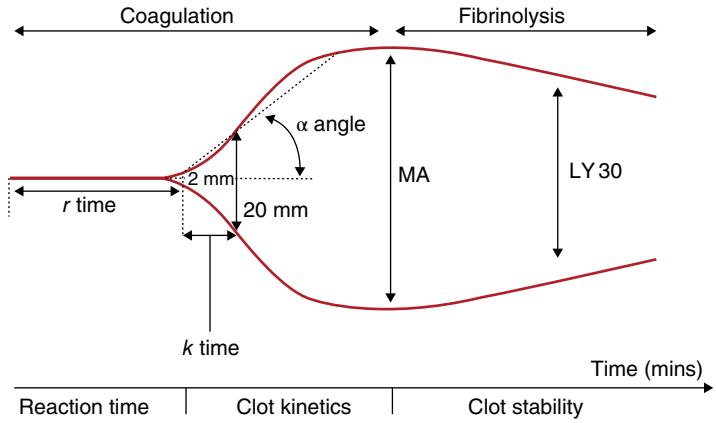

Figure 28.3 Schematic representation of thromboelastography (TEG). 1. The R value (clotting time) represents the time it takes to initiate clot formation. It is a reflection of coagulation factor activity. 2. The alpha angle represents the thrombin burst and conversion of fibrinogen to fibrin. 3. The MA is maximal amplitude or clot strength derived from platelet function. 4. The K value (kinetics of clot formation) is the time from the end of R until the clot reaches 20 mm and represents the speed of clot formation. 5. LY30 lysis time measures the degree of fibrinolysis.

Haemophilia A is classified into mild, moderate or severe (Table 28.3). The effective level for haemostasis is generally about 25–30% of factor level. Female carriers usually have > 50% factor VIII activity, but some female carriers may be symptomatic. Laboratory findings in haemophilia A include:

- Prolonged aPTT, which
  - Corrects in a 1 : 1 mixing study if no inhibitor is present.
  - Fails to correct in a 1 : 1 mixing study if an inhibitor is present.
- Decreased factor VIII activity level.

Table 28.3 Clinical manifestations and treatment of haemophilia A and B.

Factor level (% normal)	Clinical manifestation	Treatment
< 1% (severe disease)	Usual age of onset < 1 year Spontaneous bleeding common (haemarthrosis, muscle haematoma, haematuria) Bleeding post surgery and dental extraction Posttraumatic bleeding Crippling joint deformity if inadequate treatment	Regular factor VIII or IX replacement therapy (prophylaxis) Factor VIII or IX to treat bleeds Factor VIII or IX for surgery or invasive procedures +/– antifibrinolytics (tranexamic acid)
1–5% (moderate disease)	Usual age of onset < 2 years Occasional spontaneous bleeding Bleeding post surgery and dental extraction Posttraumatic bleeding	Some patients may need regular factor VIII or IX prophylaxis Factor VIII or IX to treat bleeds Factor VIII or IX for surgery or invasive procedures +/– antifibrinolytics (tranexamic acid)
6–40% (mild disease)	Usual age of onset > 2 years Bleeding post surgery and dental extraction Posttraumatic bleeding Spontaneous bleeding is rare	Regular prophylaxis usually not required Treatment of haemorrhages or cover for surgery/invasive procedures: Haemophilia A: DDAVP, tranexamic acid, factor concentrate Haemophilia B: tranexamic acid, factor concentrate

Management of patients with haemophilia A includes prevention and management of bleeding, physical therapy and rehabilitation, management of inhibitor development and co-morbidities, and ongoing education on treatment and self-care. The mainstay of treatment is to raise the factor VIII activity sufficiently to prevent or arrest spontaneous and traumatic haemorrhages or to prevent bleeding periprocedurally. There are a number of products available, including:

- Recombinant factor VIII preparations.
- Extended half-life factor VIII products.
- Plasma-derived factor VIII concentrates (which vary in degree of purity).
- Bypass agents such as activated prothrombin complex concentrates (aPCC) in case of inhibitors.
- Subcutaneous factor substitution therapy with emicizumab.
- DDAVP (for mild disease – baseline factor VIII above 15%).
- Antifibrinolytics (tranexamic or aminocaproic acid).

Recombinant products are the product of choice to prevent spontaneous joint haemorrhages (prophylaxis), as well as in the treatment of haemorrhages, because of the lack of risk of transmission of infection [2]. Plasma-derived factor concentrates undergo donor screening and viral inactivation procedures, but transmission of some non-encapsulated viruses, such as human parvovirus B19 or prion disease, and emerging infections remains a theoretical risk. Where available, long-acting recombinant factors are preferable. For example, recombinant factor VIII Fc fusion protein has a mean half-life of 19 hours and requires administration every 3–5 days for prophylaxis in most patients [3].

Patients with moderate/severe haemophilia will require factor VIII replacement for bleeding, prior to invasive procedures, surgery, etc.

One unit of factor VIII/kg bodyweight will result in an increase in plasma factor VIII activity by 2 IU/dL or 2% of normal. The amount of factor VIII concentrate required is calculated according to the formula:

$$\text{Units of factor VIII required (IU)} = \text{weight (kg)} * \text{desired factor VIII increase (IU/dL or \%normal)} * 0.5$$

The plasma half-life of non-modified factor VIII is 8–12 hours. The peak factor level should be measured 15–30 minutes after the infusion. Repeated doses at 12-hourly intervals are usually needed. Alternatively, a continuous infusion of factor VIII can be given for surgery. For soft tissue haemorrhages, levels above 50% are generally sufficient; however, for major surgery, a preoperative level of 100% is necessary, and thereafter levels of 50–100% are sufficient for wound healing. Factor VIII activity can be measured before and after doses of concentrate to ensure appropriate levels.

Emicizumab, a bispecific antibody against factors IX and X, does not replace the missing coagulation factor VIII, but does replace its function [4]. For patients with severe haemophilia A without inhibitors, prophylaxis with emicizumab may prevent hemarthrosis and spontaneous and breakthrough bleeding. However, emicizumab is not intended to treat acute bleeding episodes. Caution is required when treating breakthrough bleeding episodes while on emicizumab due to reported cases of venous thromboembolism or thrombotic microangiopathy with concomitant administration of aPCC; recombinant factor VIIa is considered to be safer.

Mild haemophilia A may be treated with DDAVP (with or without antifibrinolytics) [2]. DDAVP (0.3 μg/kg bodyweight) is given intravenously. Hyponatraemia and water intoxication are side effects of this drug. DDAVP is not recommended for patients with cardiac failure or children under 2 years of age. DDAVP should also be used with caution during pregnancy, in the elderly and in patients with vascular disease. The response to DDAVP should be assessed prior to its use to treat bleeding or cover invasive procedures to ensure that an adequate increase in the factor VIII level is achieved. Children should generally not be given DDAVP more than once per day. In adults a second dose may be considered. With subsequent dosing, the therapeutic response decreases (tachyphylaxis) and the risk of complications rises [2].

Antifibrinolytics reduce fibrinolysis and are of particular use in patients with bleeding from mucosal surfaces, such as epistaxis, oral bleeding or menorrhagia. They are given as an adjunct to DDAVP to reduce bleeding. They should be avoided in patients with haematuria to avoid clot retention. They are usually given for 7–10 days to allow adequate healing. Treatment with antifibrinolytic agents alone is of no value in the prevention of haemarthroses in haemophilia.

Haemophilia B

Congenital haemophilia B is an X-linked recessive disorder of reduced or absent factor IX activity that accounts for 10–15% of all cases of congenital haemophilia. The clinical manifestations are similar to those of haemophilia A (Table 28.3). Characteristic laboratory findings in haemophilia B include:

- Prolonged aPTT, which
  - Corrects in a 1 : 1 mixing study if no inhibitor is present.
  - Fails to correct in a 1 : 1 mixing study if an inhibitor is present.
- Decreased factor IX activity level.

Management goals are similar to those for congenital haemophilia A. Current products for treatment include:

- Recombinant factor IX products.
- Extended half-life factor IX products.
- High-purity plasma-derived factor IX concentrates.

The product of choice is recombinant factor IX [5]. If that is unavailable, then high-purity plasma-derived factor IX concentrates should be used.

The dosage of factor IX required can be calculated according to the formula:

$$\text{Units of factor IX required (IU)} = \text{weight (kg)} * \text{desired factor IX increase (IU/dL or \%normal)} * 1.0$$

The plasma half-life of non-modified factor IX is 18–24 hours, so repeated doses are given every 12–24 hours or by continuous infusion.

Long-acting recombinant factor IX (e.g. PEGylation, Fc-fusion, albumin-fusion) is also available. The half-life of factor IX Fc fusion protein is 82 hours, with a dosing interval of 10–14 days. Patients who transitioned from prophylaxis with recombinant factor IX to prophylaxis with factor IX Fc fusion protein had fewer bleeding episodes despite reduced infusion frequency and overall factor consumption [5,6]. DDAVP is of no value in haemophilia B.

Treatment of Patients with Factor VIII or IX Inhibitors

Patients with congenital haemophilia A or B can develop inhibitory antibodies against factor VIII or (less commonly) factor IX, respectively. Inhibitor development in haemophilia A is more common in patients with severe disease (prevalence of 30%) and is often heralded by increased frequency of bleeding or loss of response to factor VIII. It is diagnosed by measuring factor VIII levels before and after a dose of factor VIII concentrate and by measuring inhibitor titres using the Nijmegen-modified Bethesda inhibitor assay. Inhibitor testing should be performed routinely at least every 12 months, before surgery and if there is a suboptimal response to factor replacement therapy. Patients with inhibitor titres < 5.0 Bethesda units/mL (BU/mL) are considered low responders; patients with inhibitor titres ≥ 5.0 BU/mL are considered high responders, since they experience an exaggerated immune response with exogenous factor VIII.

For low responders (< 5.0 BU/mL), bleeding episodes can be treated with higher doses of human factor VIII [3]:

$$\text{Units of factor VIII required (IU)} = \text{weight (kg)} * 80 * \left[\left(1 - \frac{\text{haematocrit}}{100}\right) * \text{inhibitor titre}\left(\frac{\text{BU}}{\text{mL}}\right)\right]$$

An additional 50 IU/kg above the calculated loading dose is added to achieve a measurable factor VIII activity. The factor VIII level should be measured 15 minutes after administration of the bolus, and adjustments made based on individual response.

For high responders (≥ 5.0 BU/mL), factor VIII is ineffective in controlling bleeding, and the use of recombinant FVIIa or aPCC FEIBA® (Baxter, Deerfield, IL, USA) is recommended. Treatment with bypassing agents typically consists of one dose of aPCC (75–85 units/kg) or two doses of rFVIIa (90–270 μg/kg).

Emicizumab is increasingly being used to prevent haemorrhage [4]. Eradication of inhibitors with 'immune tolerance induction' using factor VIII concentrates is successful in 70–80% of patients with severe haemophilia A, but less favourable in patients with moderate/mild haemophilia A.

Treatment strategies for factor IX inhibitors are similar to those for factor VIII inhibitors. For low responders, factor IX replacement therapy may be used. Close monitoring is needed because allergic reactions and anaphylaxis may occur in up to 50% of haemophilia B patients with inhibitors. For high responders, rFVIIa may be used to control bleeding. As aPCC contains factor IX, it may trigger an allergic response; for that reason, aPCC should be avoided in haemophilia B patients.

Von Willebrand Disease

Von Willebrand disease (vWD) is the most common inherited bleeding disorder, affecting up to 1% of the population, and is due to a quantitative and/or qualitative defect in von Willebrand factor (vWF).

vWD is classified into three types (Table 28.4) [7]. The majority of vWD variants are inherited in an autosomal dominant fashion, although type 2N and type 3 vWD are autosomal recessive disorders. Clinical symptoms vary from asymptomatic to severe bleeding, typically affecting mucosal membranes. Bleeding manifestations include epistaxis, menorrhagia and prolonged bleeding after dental extraction or other procedures. A diagnosis of vWD is typically suspected only after a haemostatic challenge or an incidental finding of a prolonged aPTT during a perioperative workup. The American Society of Hematology (ASH), International Society on Thrombosis and Haemostasis (ISTH), National Hemophilia Foundation (NHF) and World Federation of Hemophilia (WFH) 2021 guidelines for diagnosis of vWD recommend using a validated bleeding assessment tool (BAT) as an initial screening test to determine who needs specific blood testing [7]. The three most important screening assays for vWD include vWF antigen, vWF platelet-binding activity and factor VIII activity. Laboratory findings in vWD are shown in Table 28.5.

Treatment differs for the various types of vWD, and recommendations are outlined in the ASH ISTH NHF WFH 2021 guidelines on the management of vWD [8]:

- *Type 1*: DDAVP is the treatment of choice. A dose of 0.3 μg/kg bodyweight is usually given intravenously. Intranasal doses (300 μg for adults or 150 μg for children) can also be given. It is important to test an individual's response to DDAVP prior to surgical procedures. Tranexamic or aminocaproic acid is often also given, either alone for minor bleeding/procedures or in conjunction with DDAVP. Plasma-derived vWF concentrates may be required in severe type 1 vWD, and with prolonged bleeding or surgery.

Table 28.4 Types and subtypes of von Willebrand disease (vWD).

Variant	
Type 1	Autosomal dominant inheritance Partial quantitative deficiency of von Willebrand factor (vWF) Normal vWF multimer distribution Mild bleeding disorder that decreases during pregnancy, ageing
Type 2	Autosomal dominant inheritance, except for type 2N (autosomal recessive or compound heterozygous) Qualitative deficiency of vWF **Subtypes:** **2A:** reduced dimerisation or multimerisation resulting in loss of high and intermediate molecular weight vWF multimers, and reduced vWF platelet-binding activity **2B:** gain of function of GPIb binding site, resulting in loss of high molecular weight vWF multimers, reduced vWF platelet-binding activity +/- thrombocytopenia **2M:** loss of function of GPIb binding site ($2M_{PB}$) or loss of function of collagen binding site ($2M_{CB}$) with normal multimers **2N:** markedly decreased vWF affinity for factor VIII, resulting in increased clearance of factor VIII
Type 3	Autosomal recessive inheritance Severe quantitative deficiency of vWF Severe haemophilia-like bleeding disorder Up to 10% of patients may develop an inhibitor to vWF

Table 28.5 Comparative laboratory findings in types of von Willebrand disease (vWD).

Type of vWD	vWF : RCoF	vWF : Ag	VWF : RCoF/ VWF : Ag ratio	FVIII activity	vWF multimers	Comments
1	↓	↓	> 0.6–0.7	↓	Normal distribution, decreased intensity	The 2021 consensus guidelines recommend a vWF activity or antigen level of < 0.30 IU/mL regardless of bleeding, and for patients with abnormal bleeding, a vWF level of < 0.50 IU/mL
2A	↓↓	N or ↓	< 0.6–0.7	N or ↓	Loss of high and intermediate molecular weight multimers	
2B	↓↓	N or ↓	< 0.6–0.7	N or ↓	Loss of high molecular weight multimers	Exaggerated platelet aggregation response to low concentration of ristocetin
2M (platelet-binding defect)	↓↓	N or ↓	< 0.6–0.7	N or ↓	Normal distribution	Normal collagen binding activity
2N (Normandy)	N	N	> 0.6–0.7	↓↓↓	Normal distribution	Decreased vWF : factor VIII binding
3	↓↓↓	↓↓↓	N/A	↓↓ or ↓↓↓	Absent multimers, except after exogenous vWF administration	

Ag, antigen; FVIII, factor VIII; N, normal; N/A, not applicable; RCoF, ristocetin co-factor; vWF, von Willebrand factor.

- *Types 2 and 3*: virally inactivated plasma-derived vWF concentrates are required, because no recombinant vWF concentrates are available. DDAVP is not indicated in type 3 vWD because of a lack of efficacy, and is contraindicated in type 2B vWD because of increased platelet binding, with subsequent thrombocytopenia.

Other Inherited Disorders

Hereditary deficiencies of other coagulation factors are rare [9]. Of these, factor XI deficiency is particularly common among Ashkenazi Jews and is inherited as an autosomal recessive trait. There is a poor correlation between factor XI levels and bleeding tendency. If available, factor XI concentrates should be given to treat bleeding; if not, then plasma should be administered. Fibrinogen concentrates have been approved for the treatment of congenital a-, hypo- or dysfibrinogenaemia and should be used in preference to cryoprecipitate for these disorders if they are available, since they undergo viral inactivation steps. Deficiencies of factors II, V, VII, X and XIII can all be treated with plasma, but if more specific therapies are available they should be used in preference. Currently, there are specific factor concentrates for factors VII and XIII. Prothrombin complex concentrates (PCCs) contain factors II, IX and X with variable amounts of VII, and are used in conditions associated with deficiencies of one or more of these factors (e.g. reversal of warfarin anticoagulation).

Acquired Haemostatic Defects

Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) is a thrombo-haemorrhagic diathesis resulting from inappropriate activation of the haemostatic system and dysregulated thrombin generation [10]. DIC may be overt (acute, uncompensated) or non-overt (chronic, compensated). Overt DIC evolves in three stages: an initial hypercoagulable stage, followed by secondary hyperfibrinolysis, and a late-consumptive stage with consumption of procoagulant, anticoagulant and fibrinolytic factors. Non-overt DIC is associated with vascular malformations and certain malignancies and results from chronic, low-grade thrombin generation and fibrinolytic activity

The main triggering mechanism for DIC is the exposure of blood to a source of tissue factor that initiates coagulation, leading to thrombin generation and fibrin formation (Table 28.6). This may cause microthrombus formation, resulting in digital infarcts and multisystem organ failure; venous and arterial thromboembolism can also occur. Secondary activation of the fibrinolytic pathway occurs with subsequent lysis of fibrin and the formation of cross-linked complexes such as D-dimers. Raised levels of these FDPs further add to the bleeding diathesis as they inhibit the polymerisation of fibrin. In late-stage overt DIC, hepatic synthesis is unable to compensate fully for the ongoing consumption of clotting factors, so there is a reduction in their levels. In addition, a consumptive thrombocytopenia develops.

The following laboratory abnormalities are variably seen in DIC, depending on the stage:

- Thrombocytopenia.
- Anaemia, fragmented red cells (schistocytes), raised reticulocyte count.
- Increased FDPs/D-dimers.
- Increased soluble fibrin monomer complexes.
- Decreased fibrinogen levels (fibrinogen is an acute-phase reactant and may be elevated in the early stages of DIC).
- Prolonged PT and aPTT.

In order to help with the diagnosis of DIC in the clinical setting, scoring systems such as that from the ISTH have been devised (Box 28.1) [11].

Treatment of the underlying disease is the most important aspect of management of patients with DIC [10]. Obstetric emergencies should be attended to immediately.

Table 28.6 Main causes of disseminated intravascular coagulation.

Condition	Examples
Infection	Septicaemia
Malignancy	Leukaemia (especially acute promyelocytic)
	Metastatic carcinomas
Obstetric disorders	Septic abortion
	Placenta praevia and abruptio placentae
	Eclampsia Amniotic fluid embolism
Trauma	Extensive surgical trauma
	Fat embolism
Shock	Burns
	Heat stroke
Liver disease	Acute hepatic necrosis
Transplantation	Tissue rejection
Extensive intravascular haemolysis	ABO-incompatible transfusion
Envenomation	Haematoxins from spider or snake bites
Vascular abnormalities (non-overt)	Kasabach–Merritt syndrome
	Large abdominal aortic aneurysm

Box 28.1 International Society of Thrombosis and Haemostasis diagnostic scoring system for overt disseminated intravascular coagulation (DIC).

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

Order global coagulation tests (prothrombin time [PT], platelet count, fibrinogen, fibrin-related marker)

Score the test results:

- Platelet count: > 100 × 10^9/L = 0, < 100 × 10^9/L = 1, < 50 × 10^9/L = 2
- Elevated fibrin marker (e.g. D-dimer, fibrin degradation products): no increase = 0, moderate increase (< 5× upper limit of normal) = 2, strong increase (> 5× upper limit of normal) = 3
- Prolonged PT: < 3 s = 0, > 3 but < 6 s = 1, > 6 s = 2
- Fibrinogen level: > 1 g/L = 0, < 1 g/L = 1

Calculate score:

≥ 5 compatible with overt DIC: repeat score daily

< 5 suggestive for non-overt DIC: repeat next 1–2 days

Heparin anticoagulation may also be useful when DIC is complicated by microvascular thrombosis or large-vessel thrombosis, especially when DIC is identified early prior to the consumptive stage. Low-dose continuous intravenous therapy (500–1000 IU/h) is one suggested regimen that can stop the clinical sequelae of DIC by inhibiting thrombin activity. In the consumptive stage of DIC, platelet, plasma and cryoprecipitate components

and/or fibrinogen concentrates are administered with the goal of maintaining the platelet count > 50 × 10^9/L, fibrinogen > 1 g/L and PT and aPTT < 1.5 times the mean control. Administration of antithrombin concentrate in sepsis-induced DIC is controversial.

Trauma

Uncontrolled haemorrhage is responsible for 30–40% of deaths from trauma [12]. By the time the patient reaches hospital, a coagulopathy has often begun and needs to be corrected promptly to prevent further haemorrhage and allow treatment of injuries. The coagulopathy is multifactorial, with the leading causes being the following:

- Consumption of clotting factors and platelets.
- Dilution of clotting factors due to fluid resuscitation/massive transfusion.
- Acidosis leading to clotting factor dysfunction.
- Hypothermia leading to clotting factor dysfunction and reduced synthesis.
- Primary hyperfibrinolysis in the acute setting and secondary hyperfibrinolysis/DIC during recovery.
- Platelet dysfunction.

The combination of acidosis, hypothermia and coagulopathy is referred to as the 'lethal triad'. Early recognition of trauma-induced coagulopathy is imperative, but conventional coagulation assays are limited by the lengthy turnaround times and failure to incorporate the cellular contribution to normal haemostasis. Algorithms using results from VHA, such as TEG and ROTEM, have become commonplace in this setting. Blood component replacement remains the cornerstone of management.

Liver Disease

In liver disease, a hypocoagulable state may result from a number of mechanisms: reduced synthesis of coagulation factors; cholestasis and subsequent malabsorption resulting in vitamin K deficiency; and an acquired dysfibrinogenaemia [13]. The platelet count is often reduced due to hypersplenism. Laboratory abnormalities may include prolonged PT and aPTT, as well as prolonged thrombin time (TT) and reptilase time (RT) due to hypo- or dysfibrinogenaemia. Chronic liver disease is also frequently associated with accelerated intravascular coagulation and fibrinolysis, with mild–moderate elevations in D-dimers and FDPs. VHA also has clinical utility in the evaluation of bleeding in patients with liver cirrhosis.

Although coagulation abnormalities occur frequently in patients with severe liver disease, they are not always associated with bleeding. Bleeding is often precipitated by an event such as surgery or liver biopsy and is rarely attributable to the haemostatic defect alone. If there is bleeding or a high risk of bleeding, then plasma transfusion is indicated, often in large volumes. Complete normalisation of a prolonged PT is often not possible and is seldom required. The use of PCCs may be considered; however, one must be aware of the potential risks of inducing thrombosis or DIC in these patients, particularly since they already suffer from impaired clearance of activated clotting factors and reduced levels of antithrombin. Perioperative or periprocedural plasma therapy is usually guided by the international normalised ratio (INR), with a typical goal of achieving an INR less than 1.6. Since thrombocytopenia and platelet function defects are also a feature of hepatic disease, platelet concentrates may also need to be given to maintain a platelet count above 20 × 10^9/L for minor surgical procedures and above 50 × 10^9/L prior to major surgical procedures or in bleeding patients. Thrombopoietin (TPO) stimulators (e.g. avatrombopag) have been demonstrated to decrease the need for platelet transfusion in patients with chronic liver disease.

Uraemia

The haemostatic defect in uraemia is mainly due to platelet dysfunction and a defect in platelet–vessel wall interactions, but laboratory abnormalities of platelet function do not

appear to correlate well with clinical bleeding. It is also thought that plasma from uraemic patients contains an inhibitor that interferes with normal vWF–platelet interaction. Dialysis is useful in reversing the haemostatic defects in uraemia, although it may not correct them entirely. Infusions of DDAVP (0.3 μg/kg) have been used successfully to provide short-term correction of the bleeding time and decrease symptoms of bleeding [14].

Complications of Anticoagulant and Thrombolytic Drugs

Vitamin K Antagonists

Warfarin decreases the biological activity of factors II, VII, IX and X, as well as proteins C and S.

Management of excessive anticoagulation depends on the INR level and whether there is minor or major bleeding [15]. In the absence of haemorrhage, warfarin should be stopped for a few days and restarted when the INR falls into the desired range. Small doses of vitamin K (1–2.5 mg) may be given orally if the INR > 5.0, as there is a significantly greater risk of serious haemorrhage at this level. If the patient is bleeding, the anticoagulant effect should be reversed. Vitamin K 5–10 mg should be given intravenously and will have an initial onset of action after 4–6 hours. The action of vitamin K is, however, not maximal for at least 24 hours, and therefore additional measures may be required:

- PCCs: Kcentra® (CSL Behring, King of Prussia, PA, USA), Beriplex® (CSL Behring) and Octaplex® (Octapharma, Lachen, Switzerland), which contain factors II, VII, IX and X, are now recommended as the first line for warfarin reversal in case of life- or limb-threatening bleeding. Dosing depends on patient INR at the time of bleeding and the PCC used. The disadvantage of these concentrates is that they carry the potential risk of inducing thromboembolism; however, the risk of thromboembolism is much lower than that with activated PCC.
- In the absence of PCCs, plasma (15–20 mL/kg) will immediately supply the necessary coagulation factors. However, very large amounts of plasma may need to be infused to correct the coagulopathy, which may lead to volume overload.

Direct Oral Anticoagulants

Several direct oral anticoagulants have been introduced that have the advantage of not requiring routine monitoring. Drugs currently available include the oral direct thrombin inhibitor (DTI) dabigatran and the direct factor Xa inhibitors rivaroxaban, apixaban and edoxaban. Idarucizumab (Praxbind®, Boehringer Ingelheim, Ridgefield, CT, USA), a humanised monoclonal antibody fragment (Fab), has been approved for reversal of the anticoagulant effect of dabigatran in life-threatening or uncontrolled bleeding or for emergency surgery or procedure. Andexanet alfa (andexanet) is a specific reversal agent that is designed to neutralise the anticoagulant effects of factor Xa inhibitors. The drug-calibrated dilute thrombin time or ecarin clotting time/chromogenic assay may be used to monitor oral DTIs, and the drug-calibrated anti-Xa assay can be used to monitor direct factor Xa inhibitors in special circumstances [16].

Thrombolytic Agents

These agents generally cause a state of systemic fibrin(ogen)olysis. Haemorrhage complicating these agents is most commonly local (e.g. at the site of catheterisation in the groin); however, intracranial or gastrointestinal bleeding may occur. Most agents have a short half-life (minutes) and so the fibrinolytic state will reverse within a few hours of drug cessation. In the case of life-threatening haemorrhage, the American Heart Association (AHA) recommends the transfusion of 10 units of cryoprecipitate and 1–2 doses of platelets, with plasma or PCC if indicated [17]. Antifibrinolytic agents may provide additional benefit.

Cardiopulmonary Bypass

Haemostatic disturbances that occur during cardiopulmonary bypass (CPB) are multifactorial and related to large doses of heparin, haemodilution, activation and consumption of platelets and coagulation factors by plastic tubing of the CPB circuit, increased thrombin generation and fibrinolysis. The use of TEG or ROTEM may help in guiding replacement therapy in bleeding patients, may improve the use of blood products and factor concentrates, and is reported to be cost-effective [18].

Thrombotic Thrombocytopenic Purpura

Patients with thrombotic thrombocytopenic purpura (TTP) require therapeutic plasma exchange (TPE) with plasma to induce platelet recovery. Caplacizumab, a nanobody targeting vWF, has been approved for management of acute acquired TTP in combination with TPE; there is an increased risk of bleeding associated with caplacizumab due to its mechanism of action [19].

KEY POINTS

1) Clinical history is the most important component in evaluation of patients with bleeding symptoms.
2) Initial laboratory investigation of haemostasis should include a full blood count with platelet count, prothrombin time, activated partial thromboplastin time and blood film.
3) In inherited bleeding disorders, recombinant products should be used where available.
4) The mainstay of disseminated intravascular coagulation treatment remains management of the underlying cause. In bleeding patients, prompt administration of fresh frozen plasma and cryoprecipitate with regular laboratory monitoring is required.
5) Appropriate guidelines (e.g. those provided by the British Committee for Standards in Haematology) should be followed when managing major haemorrhage, aiming for the following parameters: haemoglobin (Hb) > 8 g/dL; platelets > 50 × 10^9/L; prothrombin time (PT) and activated partial thromboplastin time (aPTT) < 1.5 × mean control; fibrinogen > 1 g/L.
6) Prothrombin complex concentrates should be the first choice for urgent reversal of vitamin K antagonists in cases of life- or limb-threatening bleeding.
7) Patients with thrombotic thrombocytopenic purpura should receive therapeutic plasma exchange (TPE). Simple infusion of plasma should be initiated if TPE is not readily available.

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29 Point-of-Care Haemostasis Testing

Katrina M. Morgan, Matthew D. Neal, Louis Alarcon and Christine M. Leeper

University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Conditions resulting in haemostatic derangements are prevalent throughout medical and surgical subspecialties. Rapid detection of coagulopathy is essential in order to guide management and limit morbidity and mortality, especially as the affected patient population is often critically ill. Inherent in the ability to manage derangements in haemostasis is the rapid, accurate measurement and interpretation of coagulation parameters. Point-of-care (POC) testing was developed in order to expedite detection and treatment of coagulopathy, and has become widely prevalent in the trauma and perioperative setting.

This chapter provides an overview of the POC technologies relevant to all medical fields, and especially for transfusion medicine specialists, surgeons and intensivists. We will highlight the use of POC testing in guiding trauma and perioperative resuscitation in both adults and children.

Limitations of Conventional Coagulation Testing

Conventional coagulation tests (CCTs) include assays such as prothrombin time (PT), international normalised ratio (INR), partial thromboplastin time (PTT), fibrinogen and platelet counts. These plasma-based tests were initially designed for and are useful in detecting deficiencies in coagulation factors and platelet number, as well as directing changes in anticoagulant dosing. The benefits of CCTs include their familiarity and universal use. Disadvantages include a relatively long processing time of 30–90 minutes, limiting the ability to effectively guide resuscitation in response to the rapid, dynamic changes seen in severely coagulopathic or haemorrhaging patients. Some centres have implemented rapid CCTs to expedite results in bleeding patients, with results available within 15 minutes [1]. Importantly, CCTs are static tests that fail to capture the contributions of the endothelium and cellular components of whole blood. Furthermore, CCTs offer no means to evaluate clot strength or fibrinolysis. In response to these limitations, many clinicians incorporate POC tests to evaluate and manage abnormalities in haemostasis.

Point-of-Care Testing Options

Multiple POC tests have been developed for use in diverse clinical situations. The ideal POC test is readily accessible to clinicians, easy to use, and produces accurate and

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reliable results. New technology is emerging with the goal of developing smaller, portable testing platforms to occupy minimal space and be resistant to ambient conditions, allowing for use in prehospital and other bedside settings. While no single POC test yet serves as a gold standard, there is more widespread availability of POC tests and increasing data to support evidence-based practice and guide test selection.

In this chapter, we will describe various POC tests and how to interpret and subsequently apply the results in order to guide patient management in the acute care setting. We will specifically discuss viscoelastic testing, including thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®), and tests that analyse platelet function.

Viscoelastic Testing

Viscoelastic monitoring (VEM) utilises a sample of whole blood and reports the kinetics of clot formation in real time. This includes time to clot formation (fibrin cross-linking), maximal clot strength (platelet function) and clot breakdown (fibrinolysis). Results are available within minutes and can be displayed on hospital-wide computer systems for remote viewing capabilities. Rapid results allow for accurate assessment and specific treatment of coagulopathy, which is particularly essential in critically ill surgical and trauma patients whose haemodynamics and location of treatment change rapidly. Additionally, VEM captures the contribution of endothelium and cellular components within blood, monitors all phases of coagulation, measures fibrinolysis and can provide information on hypercoagulability, which are not all found in traditional coagulation tests.

Two main VEM technologies, TEG and ROTEM, will be described in more detail here. Both tests are similar in variable measures and mechanics of evaluation, with tracings that depict similar haemostatic functions (Table 29.1 and Figure 29.1). Overall, TEG and ROTEM are essentially equivalent in terms of performance. However, studies show weak or no association between the two tests, limiting direct comparison of the various parameters. This may be related to the assays using different coagulation triggers [2]. Current assays available for TEG/ROTEM are listed in Tables 29.2 and 29.3.

For both manufacturers, samples are collected in citrated tubes and processed soon after collection per manufacturers' standards to maintain the integrity of the specimen. TEG and ROTEM both use a semi-automatic pipette to place citrated whole blood and different activators into plastic cups. After recalcification, coagulation is initiated by tissue factor (EXTEM, RapidTEG) or by a contact activator (INTEM, KaolinTEG). Once thrombin is generated in the blood, platelets are activated to express activated glycoprotein (GP) IIb/IIIa receptors, and fibrin is formed and polymerised. The interactions of GPIIb/IIIa receptors and polymerised fibrin increase the viscoelasticity of the blood, and the increased torque is detected optically (ROTEM) or mechanically (TEG) and displayed on a screen (Figure 29.2). The addition of heparinase neutralises heparin in the blood sample (HEPTEM and Kaolin TEG with heparinase), and cytochalasin D results in inhibition of the platelet cytoskeletal reorganisation, thus inhibiting fibrin binding to GPIIb/IIIa (FIBTEM assay).

Cartridge-Based Technology

A TEG cartridge-based test has recently been developed called TEG6s. The cartridge-based system works by automating all sample aliquoting, reagent mixing and testing. A pneumatically controlled microfluidic cartridge takes initial blood samples and divides them across four distinct test channels. Each test channel includes a set of reagents to conduct four different assays simultaneously. The assays available include Kaolin TEG with or without heparinase, RapidTEG, Functional Fibrinogen and Platelet Mapping. TEG6s works by exposing coagulating blood to

Table 29.1 Comparison of TEG and ROTEM parameters.

Haemostatic function	Blood component responsible	TEG parameter	ROTEM parameter	Parameter reflects	Recommended treatment
Clot initiation	Clotting factors	R/ACT	CT	Time to designated clot formation (2 mm deflection above baseline)	FFP, PCC
Clot propagation	Fibrinogen	K time	CFT	Time to designated clot firmness (from 2 mm above baseline to 20 mm above baseline)	Fibrinogen (cryoprecipitate)
	Fibrinogen	α angle	α angle	Rate of increase in clot formation (tangent line from point of clot initiation [R/ACT or CT] to slope of developing curve)	Fibrinogen (cryoprecipitate)
Clot strength	Platelets	MA	MCF	Maximum clot strength (peak amplitude of tracing)	Platelets, cryoprecipitate, DDAVP
Fibrinolysis	Multiple	LY30	LI	Clot degradation TEG: % reduction in AUC 30 min after MA ROTEM: % clot remaining compared to MCF 30 min after clot initiation	Antifibrinolytics: tranexamic acid, aminocaproic acid

ACT, activated clotting time; AUC, area under the curve; CFT, clot formation time; CT, clotting time; DDAVP, desmopressin; FFP, fresh frozen plasma; LI, lysis index; LY30, lysis fraction at 30 minutes; MA, maximum amplitude; MCF, maximum clot firmness; PCC, prothrombin complex concentrate; R, reaction time; ROTEM, rotational thromboelastometry; TEG, thromboelastography.

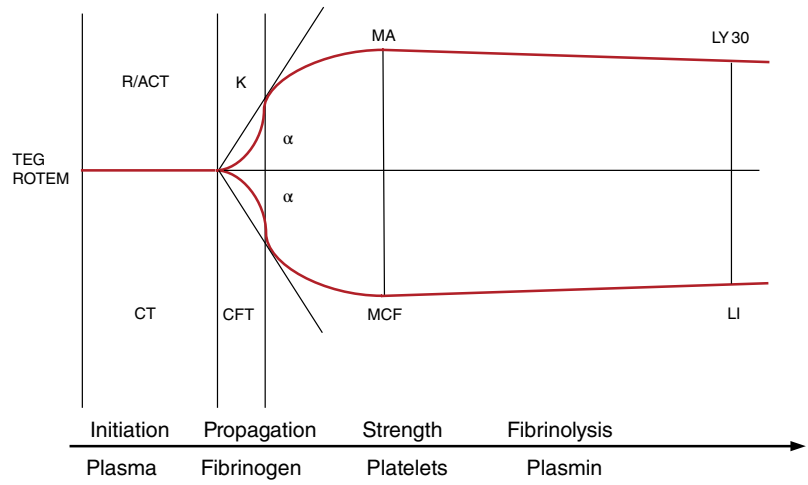


Figure 29.1 Coagulation parameters measured by thromboelastography (TEG)/rotational thromboelastometry (ROTEM). Clot initiation is measured as time to deviation from baseline by 2 mm in TEG/rTEG (reaction time [R]/activated clotting time [ACT]) and ROTEM (clotting time [CT]). Clot propagation is measured as the time for clot amplitude to reach from 2 to 20 mm by K time (TEG) and clot formation time (CFT; ROTEM) and the angle of divergence from baseline for both TEG and ROTEM (α). Maximal clot strength is measured as the distance of tracing deviation from baseline for both TEG (maximum amplitude [MA]) and ROTEM (maximum clot firmness [MCF]). Fibrinolysis is measured as percent decrease in amplitude at 30 minutes for TEG (lysis fraction at 30 min [LY30]) and as the percent of maximal amplitude remaining at 30 minutes for ROTEM (lysis index [LI]).

Table 29.2 TEG® 5000 system assays (Haemonetics Corp., Braintree, MA, USA).

TEG® Test	Components	Description
Kaolin TEG	Kaolin activator	Intrinsic pathway-activated assay, similar to aPTT
Kaolin TEG with Heparinase	Kaolin activator and lyophilised heparinase	Eliminates the effect of heparin. Can be used in combination with Kaolin TEG to assess the effect of heparin
RapidTEG	Tissue factor and kaolin	Extrinsic and intrinsic pathways to speed up coagulation process and rapidly assess coagulation properties
TEG Functional Fibrinogen	Tissue factor and abciximab (GPIIb/IIIa inhibitor)	Extrinsic pathway with platelet inhibitor to restrict platelet function. Allows for quantification of fibrinogen contribution to clot strength
TEG Platelet Mapping	ADP or arachidonic acid	Utilises a platelet receptor-specific tracing in conjunction with Kaolin TEG. Identifies level of platelet inhibition and aggregation

ADP, adenosine diphosphate; aPTT, activated partial thromboplastin time; GP, glycoprotein.

Table 29.3 ROTEM® delta system assays (Werfen, Bedford, MA, USA).

ROTEM test	Components	Description
INTEM	Ellagic acid activator	Contact activation with phospholipid and ellagic acid; similar to aPTT
EXTEM	Tissue factor	Tissue factor activation; similar to PT
HEPTEM	Ellagic acid activator and lyophilised heparinase	Heparinase to neutralise heparin. Used in conjunction with INTEM to assess heparin effect
APTEM	Aprotinin	Inhibits fibrinolysis. Used in conjunction with EXTEM to assess fibrinolysis
FIBTEM	Tissue factor and cytochalasin D	Blocks platelet contribution to clot formation. Allows for quantification of fibrinogen contribution to clot strength

aPTT, activated partial thromboplastin time; PT, prothrombin time.

vibrational frequencies. The resonant frequency changes with clot strength, enabling the coagulation process to be depicted in the same way as the TEG 5000 device. Despite technological differences between the two devices, studies have shown that they provide similar results [3].

ROTEM sigma is the POC cartridge-based system-equivalent test for ROTEM delta. Mechanically, ROTEM sigma is compatible with previous ROTEM devices. ROTEM sigma has two cartridges, each providing FIBTEM, EXTEM, INTEM and APTEM tests, with or without heparinase. ROTEM sigma is approved for use in Europe and publications show comparable functions to the previous generations of thromboelastometry devices [4].

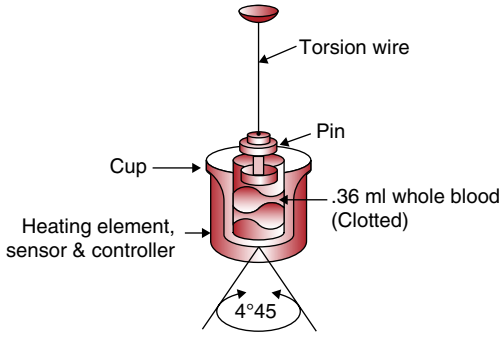


Figure 29.2 Systematic representation of TEG 5000. Whole blood is placed into a cup, a pin on a wire is suspended in the blood, and the cup rotates in alternating directions to simulate venous flow. As clotting occurs, fibrin formed between the pin and cup creates a rotational force on the pin, which is measured via a torsion wire and an electromagnetic transducer. The readout reaches a maximum value once maximum clot strength occurs, and converges back to baseline with the onset of clot lysis. ROTEM delta utilises a similar approach to TEG 5000 with cups and pins; the cup is stationary and heated, and a rotational force is applied to the pin. A collimated light beam from a light emitting diode (LED) is reflected off a mirror coupled to the pin, and the motion is tracked by a photosensor. *Source*: Reproduced with permission from Hemoscope Corporation Niles, IL, USA.

Use of Point-of-Care Tests in Trauma

Adults

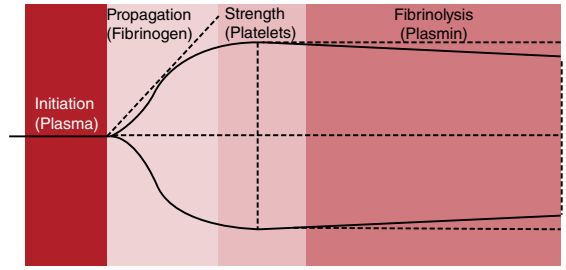
Trauma is a leading cause of mortality worldwide, and haemorrhage represents the primary cause of preventable death after injury. Trauma resuscitation focuses on control of haemorrhage, administration of haemostatic agents and mitigating trauma-induced coagulopathy (TIC). TIC is associated with increased functional disability and mortality in paediatric and adult trauma patients. Haemostatic resuscitation involves early and aggressive administration of balanced component products or whole blood, as well as adjuncts such as tranexamic acid. The transfusion practice immediately after injury is often empirical, as this begins in the prehospital setting and resuscitation bay without results of clinical laboratory tests to serve as a guide.

Targeted and individualised resuscitation guided by serial markers of coagulopathy follows or supplements this empirical balanced resuscitation strategy. CCTs have been used in this treatment algorithm, but due to limitations described above VEM is now preferred for this purpose in many centres. VEM allows for rapid assessment of the patient's response or resistance to blood product administration in critical patients. Studies have also shown that TEG-guided resuscitation leads to shorter length of hospital stay, improved cost-effectiveness and decreased blood product administration [5,6].

VEM-directed trauma resuscitation algorithms rely upon serial testing: the first test is obtained and the first round of product transfusion occurs, followed by repeat VEM to evaluate for response to the product and any additional product requirements. This cycle continues until the patient stabilises or has no additional testing or transfusion needs. A multinational, prospective observational study, TACTIC ('Targeted Action for Curing Trauma-Induced Coagulopathy'), developed data-driven thresholds for blood product resuscitation in trauma patients, shown in Figure 29.3 [7].

Subsequently, a multicentre randomised controlled trial (ITACTIC, 'Implementing Treatment Algorithms for the Correction of Trauma-Induced Coagulopathy') compared outcomes in injured patients who received an empirical massive transfusion protocol supplemented by haemostatic therapy guided by VEM (TEG6S and ROTEM sigma) against a standard massive transfusion protocol (MTP) augmented by active CCT monitoring and guided interventions. There were no differences in mortality between CCT and VEM-guided therapy; however, subjects in the VEM group received targeted product transfusion 1.8 times more frequently than those in the CCT group [7]. Another systematic review and meta-analysis conducted by the Eastern Association for the Surgery of Trauma (EAST) recommended using TEG/ROTEM-guided

(a)



(b)

	Plasma (4 units)	Fibrinogen (4 g)	Platelets (1 pack)	Tranexamic acid (1 g)
TEG	rTEG MA ≥ 65 mm AND rTEG ACT > 120s	FF TEG MA < 20 mm	(rTEG MA - FF TEG MA) < 45 mm	rTEG Ly30 > 10%
ROTEM	EXTEM CA5 ≥ 40 mm AND EXTEM CT > 80 s	FIBTEM CAS < 10 mm	(EXTEM CAS – FIBTEM CAS) < 30 mm	LI30 > 85%
CCT	INR > 1.2 AND Fibrinogen ≥ 2 g/L	Fibrinogen < 2 g/L	Platelets < 100 × 10^9c/L	No indication

Figure 29.3 Data-driven thresholds for component resuscitation in trauma resuscitation. (a) A colour-coded viscoelastic monitoring (VEM) tracing is depicted with the four phases of clot dynamics measured by thromboelastography (TEG) and rotational thromboelastometry (ROTEM), accompanied by the primary biological component responsible for the measured activity. (b) The table outlines thresholds for additional component resuscitation based on VEM and CCT, adapted from Baksaas-Aasen et al. [7]. The quantity of additional component to be administered is shown in parentheses. ACT, activated clotting time; CA5, clot amplitude at 5 minutes; CT, clotting time; EXTEM, extrinsic rotational thromboelastometry; FF TEG, Functional Fibrinogen thromboelastography; FIBTEM, fibrin-based rotational thromboelastometry; INR, international normalised ratio; LI30, unlysed fraction at 30 minutes; Ly30, lysis fraction at 30 minutes; MA, maximum amplitude; rTEG, rapid thromboelastography. *Source*: Sayce AC, Neal MD, Leeper CM. Viscoelastic monitoring in trauma resuscitation. Transfusion 2020;**60**(Suppl 6):S33–51. Reproduced with permission of John Wiley & Sons.

resuscitation strategies in adult trauma patients with ongoing haemorrhage and concern for coagulopathy in order to reduce product transfusions and mortality, though with a low level of evidence [8]. Additional studies are needed to identify specific injury types and physiologies that may benefit from targeted transfusion.

Children

Injury is the most common cause of mortality in young adults and children, accounting for 75% of all paediatric deaths. Injury patterns are unique in children compared to adults, with less haemorrhagic shock and penetrating trauma, and high morbidity due to traumatic brain injury. Paediatric resuscitation strategies emphasise bleeding cessation, damage control resuscitation and limiting TIC. Severely injured children experience coagulation dysregulation that can manifest as a tendency towards both hypo- and hypercoagulable states. These states are associated with poor outcomes, including increased mortality, length of hospital stay, transfusion requirements and organ failure [9].

A growing body of literature supports utilisation of VEM in the assessment and treatment of paediatric trauma patients; despite this, a survey of providers caring for paediatric trauma patients showed that only 31% of respondents used VEM regularly, despite its being available to 63%. Reasons for not using VEM-guided resuscitation included limited paediatric-specific data, unfamiliarity with the interpretation of TEG/ROTEM and lack of appropriate laboratory resources to run the tests. Additionally, many paediatric trauma centres rely on adult ranges for VEM-guided resuscitation. Some of the available studies on the use of VEM in paediatric trauma patients are highlighted below.

Table 29.4 Example of TEG-based transfusion algorithm used at a level 1 paediatric trauma centre.

TEG parameter	Normal range	Transfusion trigger	Product	Dose
ACT	86–118 s	> 128 s	Plasma	20 mL/kg
α angle	64–80 degrees	< 60 degrees	Cryo	1 unit/10 kg
K time	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-year-old (adult dose): 1 g loading dose over 10 min followed by 1 g infusion over 8 h ≤ 12-year-old: 15 mg/kg (max dose of 1 g) loading dose over 10 min followed by 2 mg/kg/h infusion over 8 h

ACT, activated clotting time; cryo, cryoprecipitate; LY30, lysis fraction at 30 minutes; MA, maximum amplitude; TXA, tranexamic acid.

In an early study on the use of TEG in paediatric trauma patients, activated clotting time (ACT), K time and α angle correlated with activated partial thromboplastin time (aPTT), and maximum amplitude (MA) correlated with platelet count. These values independently predicted the need for packed red blood cell and plasma transfusion within 6 hours. Additionally, all RapidTEG values, except LY30, predicted mortality [10]. Multiple TEG parameters are commonly abnormal in severely injured children; further, dysregulation of clot strength, fibrinolysis and clotting factors have been shown to be associated with increased mortality, thrombotic events, transfusion requirements and mortality in severely injured children [9]. TEG has also been used to characterise fibrinolysis phenotypes after injury in children. In a cohort of paediatric trauma patients, 19.6% had a hyperfibrinolysis evident on admission TEG, while 38.3% had fibrinolysis shutdown phenotype. These abnormalities correlated with mortality and the need for blood transfusion [11].

Few protocols for VEM-directed resuscitation have been developed for children. Studies have shown decreased blood product administration and more rapid recovery of coagulation profiles in paediatric patients managed with ROTEM-directed transfusion [12]. An example of a TEG-based protocol for transfusion triggers based on the American College of Surgeons' Trauma Quality Improvement Program (ACS TQIP) guidelines is listed in Table 29.4.

Use of Point-of-Care Tests in Surgical Bleeding

Cardiac Surgery

Bleeding is a common complication after cardiac surgery, leading to anaemia, increased blood product usage, pericardial tamponade, surgical re-exploration and potentially death. Therapeutic and prophylactic transfusion of blood products has been used for decades in cardiac surgery; however, these resuscitative techniques can be associated with increased morbidity and mortality, especially if they are administered inappropriately. In cardiac surgery, the use of POC TEG is associated with decreased transfusion requirements in adults and children. In children, it is also associated with shorter mechanical ventilation and intensive care unit length of stay. However, VEM has not been associated with decreased mortality or postoperative bleeding in either population [13].

ROTEM has also been extensively used to investigate the role of fibrinogen concentration and identifying a possible trigger point for fibrinogen supplementation in children undergoing cardiac surgery. In the Albumin versus Plasma for PaEdiAtric pRiming (APPEAR) study, ROTEM showed a significant increase in FIBTEM and EXTEM maximum clot firmness (MCF) related to higher fibrinogen concentrations, which was associated with a significant reduction in bleeding time in this paediatric population. There was also an association with low post-protamine FIBTEM MCF and postoperative bleeding [13]. Given these findings, use of VEM is recommended to guide haemostatic transfusion in paediatric cardiac centres.

Liver Transplant Surgery

Thromboelastography was initially popularised for use in orthotopic liver transplantation (OLT), since that is a complex procedure with great potential for bleeding in patients with cirrhosis and baseline coagulation changes. There has been significant progress in reducing perioperative blood loss over the years, and intraoperative monitoring of coagulation has become fundamentally important. A randomised clinical trial on the use of TEG during OLT found that TEG-guided resuscitation decreased transfusion of fresh frozen plasma (FFP), and there was a trend towards decreased blood loss in the TEG-monitored patients; however, there were no differences in three-year survival [14]. A more recent prospective study showed similar findings, with decreased use of FFP and antifibrinolytics in a ROTEM-guided transfusion group compared to CCT-guided transfusion in patients with chronic liver disease undergoing OLT. The ROTEM group had significantly more transfusions with fibrinogen, which reduced the need for transfusion of other blood products [15].

Other Surgery Populations

With increased availability, familiarity and visibility in recent years, VEM has been utilised by many clinicians for management of transfusion and coagulopathy across a wide range of surgical specialties. Additional study is required to provide specialty-specific evidence-based recommendations. However, a recent meta-analysis on the use of TEG/ROTEM-guided resuscitation strategies compared to non-TEG/ROTEM strategies in adult surgical patients included general surgery, orthopaedic surgery and transplant surgery patients. The effect of TEG/ROTEM was inconsistent across several outcomes studied, including blood transfusions, the need for angioembolic, endoscopic or surgical intervention, and mortality. However, given the potential benefit of fewer patients being exposed to blood transfusions and decreased blood product utilisation combined with no harm to the patient using a TEG/ROTEM-guided strategy, a VEM-guided resuscitation strategy was recommended in all adult surgical patients with ongoing haemorrhage and concern for coagulopathy [8].

Supplemental Assays

Supplemental assays have been developed by both TEG and ROTEM. In the Functional Fibrinogen test for TEG, kaolin and a monoclonal GPIIB/IIIa receptor antagonist are added to whole blood to hinder the interaction of fibrin and platelets, thus allowing one to determine the contribution of fibrinogen to clot strength. Additionally, similarly to Plateletworks, TEG/ROTEM Platelet Mapping assesses platelet response to agonists, and these assays have been shown to correlate well with the gold-standard assays for platelet function, impedance and light transmission aggregometry. Platelet Mapping is by far the best studied of these assays.

Analysis of Platelet Function

Several POC platelet tests exist and attempt to capture the complex *in vivo* processes involving platelet function, including platelet

aggregation on injured vessel walls, platelet activation, shape change stimulated by endogenous agonists like adenosine diphosphate (ADP) or thromboxane A1, and binding of fibrin(ogen) to GPIIb/IIIa receptors. Due to this complexity, one test is unable to capture all platelet functions, including the impact of high flow, shear and locally generated thrombin. Therefore, over time several POC tests have been developed and increasingly used in clinical settings. Broadly, these assays introduce an agonist to a whole blood sample in the setting of a shear stress, and quantify subsequent aggregation. Platelet Function Analyser (PFA)-100 (Siemens Diagnostic Deerfield, IL, USA) was developed to mimic bleeding time; VerifyNow (Accumetrics, San Diego, CA, USA) measures platelet agglutination with fibrinogen-coated beads; Plateletworks (Helena Laboratories Beaumont, TX, USA) measures platelet count before and after addition of a platelet agonist (ADP or arachadonic acid); and Multiplate (Dynabyte, Munich, Germany) uses impedance aggregometry to measure increases in impedance due to platelet aggregation.

POC platelet function testing has been used in multiple clinical scenarios, including cardiac surgery, postcardiac catheterisation and trauma. In cardiac surgery, multiple early studies found that POC platelet function tests predicted blood loss, reliably guided perioperative platelet transfusions and decreased transfusion requirements in cardiac surgery. Contrary to this, a recent meta-analysis was unable to demonstrate the predictive accuracy of commonly used POC platelet function devices, though a noted limitation of this work was methodological and measurement heterogeneity, which limited the validity of the data synthesis [16]. Data in the paediatric cardiac surgery population are even more limited, though one study reported an association between low preoperative platelet function and increased blood product transfusion [17].

In trauma, platelet response is impaired in injured subjects compared to controls, and abnormal POC platelet response reliably predicts the need for platelet and red blood cell transfusion [18]. Many studies report an association between platelet impairment and traumatic brain injury, as well as an association between abnormal POC platelet tests and mortality [19]. Importantly, these assays do not always correlate with injury severity and may overestimate the degree of impairment in those with minor injury [20]. Furthermore, there are limited data addressing the issue of whether platelet transfusion to reverse the dysfunction seen on these POC assays results in improved patient outcomes; study in these areas is warranted and ongoing.

POC platelet function tests have additionally been utilised in the postprocedural setting to measure platelet reactivity to antiplatelet medications. Several randomised studies, including the ARCTIC study, have been unable to show clinical superiority in using platelet function monitoring to adjust therapy in patients undergoing coronary stenting. Additionally, in a randomised controlled superiority trial (ANTARCTIC), elderly patients with acute coronary syndrome had similar ischaemic and bleeding rates whether they were treated with conventional therapy (low-dose prasugrel) or with platelet-function test-guided prasugrel escalation or de-escalation. Therefore, POC platelet function tests are currently not recommended in this clinical scenario [21]. In summary, these studies highlight the fact that while POC platelet tests can offer important information and prognostication value, overinterpretation should be avoided and clinical judgement prioritised, given the difficulty of simulating the multifactorial properties of platelets *ex vivo*.

Conclusion

In summary, POC testing has evolved into an efficient and effective alternative to CCTs for diagnosing, analysing and treating coagulopathy. Transfusion algorithms have been developed in adult and paediatric trauma patients that rely on the results of

TEG/ROTEM in order to guide resuscitation, which has been associated with decreased use of blood products and, in some studies, decreased mortality. Additionally, in adult and paediatric patients with surgical bleeding, studies have shown that the use of POC VEM is also associated with decreased blood product utilisation. Thus, it is recommended to have and utilise TEG/ROTEM during perioperative monitoring, especially in cardiac surgery and during liver transplants.

KEY POINTS

1) Routine conventional coagulation tests (prothrombin time/international normalised ratio, partial thromboplastin time, fibrinogen, platelet counts) are limited by long processing time and inability to measure several important aspects of haemostasis; some centres utilise rapid conventional coagulation testing to expedite results in bleeding patients.
2) Viscoelastic tests (thromboelastography [TEG] and rotational thromboelastometry [ROTEM]) are whole blood assays that include cellular and endothelial contributions to coagulation; these tests allow for rapid assessment and personalised treatment of coagulopathy in critically ill patients.
3) Current adult and paediatric trauma transfusion algorithms rely on TEG/ROTEM, and studies have shown decreased product administration when these algorithms are utilised.
4) In perioperative adult and paediatric patients at a high risk of bleeding and coagulopathy, TEG/ROTEM-guided resuscitation has been recommended to decrease blood product administration.
5) Point-of-care platelet function tests have been developed to measure platelet dysfunction and therapeutic responses to antiplatelet therapy. However, more study is required to clarify the clinical utility of these tests, given the difficulty of simulating the multifactorial properties of platelets *ex vivo*.

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30 Transfusion in Bleeding Patients

Aaron S. Hess[1], Lynn G. Stansbury[2] and John R. Hess[3]

[1] *Departments of Anesthesiology and Pathology and Laboratory Medicine, University of Wisconsin, Madision, WI, USA*
[2] *Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, WA, USA*
[3] *Departments of Laboratory Medicine and Pathology and Hematology, University of Washington, Seattle, WA, USA*

Bleeding is common and is associated with an array of potentially lethal pathophysiological consequences: hypovolaemia, reduced oxygen carrying capacity, decreased coagulation capacity and end-organ damage. Not surprisingly, transfusion is the most common consented procedure performed in hospitals. Likewise, the problems associated with blood transfusion – volume overload, unnecessary transfusion of red blood cells (RBCs) and dilutional coagulopathy – are common in hospital care. The goal of haemotherapy is to reap the benefits of blood for our patients while minimising its risks.

Severe bleeding is an emergency requiring systems-wide approaches to bleeding-source control and resuscitation. Cardiovascular surgery and liver transplant are planned events where anticipated bleeding may be large and escalate suddenly. Trauma, gastrointestinal (GI) bleeding and maternal haemorrhage occur suddenly and often far from blood resources. Large-volume transfusion can be associated with dilution, acidosis, hypothermia and hypocalcaemia, and must be managed in a framework of established clinical protocols and prompt, focused, cost-effective laboratory support.

This chapter will review the basics of evidence-based transfusion support for actively bleeding patients, with a particular focus on coagulation.

Volume Loss in Acute Bleeding

Normal blood volume in healthy people is about 7% of bodyweight, or about 5 L in a 70 kg individual. Healthy young people can lose 10–15% of that amount, as in the case of donating a unit of whole blood, with only mild symptoms or change in vital signs and only a proportionate, transient reduction in maximum oxygen transport capacity. With greater volume loss – and over shorter periods of time – healthy individuals tend to show a stepwise progression of compensatory signs.

On this basis, shock has been divided into four 'stages'. Stage I is 0–15% volume loss, which is generally asymptomatic in healthy young individuals. Stage II is 15–30% volume loss, associated with postural lightheadedness and tachycardia. Stage III is 30–40% volume loss, associated with supine hypotension, as volume return to the heart is

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not enough to maintain cardiac output. Stage IV is > 40% volume loss and is associated with severely reduced oxygen delivery, organ dysfunction, metabolic acidosis and death.

In otherwise healthy individuals, if blood loss is relatively slow and oxygen transport maintained, physiological homeostatic processes, including the haematopoietic system, can handle the deficits reasonably quickly: RBCs, platelets, plasma coagulation factors and albumin are replaced within hours to days. In certain kinds of injuries such as burns, the cytokine response, specifically tumour necrosis factor (TNF)α, interleukin (IL)1a and IL6, severely suppresses marrow RBC production, creating an acute form of the anaemia of chronic inflammation.

If blood loss is large enough, haemotherapy will be necessary. The goal is to maintain cardiac output and oxygen carrying capacity, and to prevent or treat coagulopathy. If patient condition permits, the cellular components, RBCs and platelets, should be given a unit at a time and their effect on haemoglobin (Hb) and platelet count documented. Anticipated responses are summarised in Box 30.1. Transfusion goals for these patients are often expressed as component-specific 'transfusion triggers' and remain areas of active debate.

Box 30.1 Results of blood product administration.

- Red blood cells: One unit increases haemoglobin by 1 g and haematocrit by 3% in a 70 kg individual.
- Platelets: One apheresis unit – or a pool of five whole blood derived units – increases the platelet count by $\approx 30 \pm 15 \times 10^9$/L in afebrile, non-obese, non-bleeding patients.
- Plasma: One 250 mL unit increases plasma coagulation factors by 2.5%. Six to eight units are needed for a 15–20% correction in critically ill or injured patients.
- Cryoprecipitate: One 5 U pool will deliver 1.5 g of fibrinogen and increase fibrinogen concentration by 0.3–0.4 g/L.

Platelet Counts

Cancer patients undergoing chemotherapy who develop bleeding with mucositis will usually stop bleeding if platelet counts can be raised to 40–50 × 10^9/L. For general surgery along anatomical planes, current guidelines suggest platelet counts of at least 50 × 10^9/L. With closed-space bleeding – eye, calvarium and spinal canal – platelet counts should be raised to 100 × 10^9/L, a point where circulating platelet numbers are enough to normalise skin bleeding times.

Assessment of Coagulation Factors

The correction of defects in plasma coagulation is complicated: there are many coagulation factors and no agreed-upon transfusion triggers. The historical answer, keeping the prothrombin time (PT) and activated partial thromboplastin time (aPTT) less than 1.5 times normal, is based on observations of spontaneous bleeding of disturbed tissue in eight profoundly injured trauma patients [1]. The PT and aPTT do not have the same implications in patients with a lower burden of injury and endothelial disruption [2]. Also, because the concentration of every coagulation factor in donor plasma is ≤ 1 IU/mL, large amounts of plasma are needed to significantly change the PT and aPTT. The 6–8 U of plasma that can be required to decrease the PT international normalisation ratio (INR) from 1.5 to 1.3 are often not tolerated in elderly individuals and can lead to transfusion-associated cardiac overload (TACO). Elevated INRs by themselves are well tolerated, with values of 3.5–5.0 considered appropriate for individuals with certain mechanical heart valves. Finally, the function of the whole plasma coagulation system is critically dependent on the concentration of fibrinogen, and the ability of the system to convert fibrinogen rapidly into stable fibrin and protect that fibrin from fibrinolysis.

Massive Blood Loss

Definitions of massive blood loss and massive transfusion are necessary to benchmark research, but have varied in scope and utility over the roughly 70 years that the adjective 'massive' has appeared increasingly commonly in the medical literature associated with blood loss and transfusion. All of the definitions involve some metric of volume over time, but hark back to the original uses of the term (in massive obstetrical haemorrhage) as the loss of at least one blood volume in less than 12 hours. With the recognition that bleeding is the most common acute but potentially reversible cause of death following injury – median time to death in these patients remains about 2 hours [3] – more recent definitions focus even more tightly on the time interval associated with volume loss [4].

Massive blood loss is not rare, occurring in 1/2000–1/4000 individuals yearly in developed countries not at war. Two recent, retrospective, population-based reviews examine massive bleeding and massive transfusion response in European and American civilian advanced-care systems [5,6]. Though their definitions of massive bleeding and massive transfusion differ slightly, together these studies suggest that cardiovascular surgery accounts for about a third of all massive bleeding/massive transfusion episodes, followed by transplant surgery (20%), particularly liver; trauma (15–16%); medical causes (10%), particularly GI bleeding; and maternal haemorrhage (2%).

Overall, population-based observations like these have led to new standards and accreditation requirements for hospitals and their blood services, and are driving efforts in trauma care to extend blood product–based resuscitation into prehospital care and to other, non-trauma causes of uncontrolled haemorrhage. Improvements in the management of massive blood loss following injury using haemostatic or 'damage control' resuscitation to support surgical or transvascular control of bleeding have, for example, reduced the mortality of grade V liver injury by 70%. Likewise, bleeding is now the third rather than the second most common cause of in-hospital trauma death in civilian populations with access to the best care.

The Acute Coagulopathy of Trauma and Massive Injury

The demonstration in 2003 of an acute coagulopathy of trauma present at trauma centre admission prior to resuscitation [7] has led to dramatic changes in thinking and emphasis in trauma resuscitation. Prolongation of PT/INR is the most frequently observed abnormality, followed by reduced fibrinogen, prolongation of aPTT and reduced platelet counts. The latter, even when normal at admission, fall precipitously in the first hour of care. The resulting coagulopathy is complicated by endothelial damage, precipitation of trauma-associated inflammatory responses, acidosis, hypothermia and hypocalcaemia. Causes of coagulopathy in trauma patients are outlined in Box 30.2. Across the spectrum of conventional coagulation tests, as the severity of anatomical injury increases, measured by increased injury severity scores (ISS), so do the prevalence and severity of coagulopathy and the likelihood of death [2].

Early recognition and treatment of the acute coagulopathy of trauma are central to current concepts of trauma care, but both remain areas of active debate and research. Early clinical diagnosis is complicated by compensatory vascular responses obscuring blood loss and the cryptic nature of much blunt injury, and laboratory support may be unavailable or delayed. Early treatment is often complicated by the lack of immediately available haemostatic blood products. Much of the thrust of research over the past 20 years has been towards life-saving interventions – products, procedures and systems changes – that can bridge this extremely high-risk early resuscitation period, roughly the first 3 hours of care.

Box 30.2 Causes of coagulopathy in trauma patients.

- Blood loss: ~30–40% of blood volume can be lost in patients with stage 3 or 4 shock before resuscitation begins.
- Blood dilution: physiological vascular refill and non-plasma fluid administration dilute plasma coagulation factors and platelets.
- Hypothermia: reduces plasma coagulation enzyme activity by 10% per degree C. Coupling between platelet adhesion and activation is lost between 30 and 34 °C.
- Acidosis: reduces coagulation factor complex assembly and, therefore, enzyme activity. Activity is 50% at pH 7.2, 30% at pH 7.0 and 20% at pH 6.8.
- Coagulation factor and platelet consumption: coagulation factors and platelet exist in the normal body in limited amounts. The amounts can be severely depleted by large endothelial area injury and local cycles of local activation and inactivation.
- Fibrinolysis: protein C inactivation of plasminogen activator inhibitor can allow clot breakdown, emphasising the importance of tranexamic acid.

Principles of Haemostatic Resuscitation

As noted above, more than half of all severely injured trauma patients who reach trauma centre care alive but go on to die of uncontrolled bleeding will be dead by the end of the first 2 hours of care. Effective response to massive bleeding is complex and requires tight, well-considered and well-practised teamwork at all levels of clinical and laboratory care. Current principles of trauma resuscitation are summarised in Box 30.3. The authors of this chapter all believe that – and come from institutions where – this starts with prehospital and emergency department emphasis on early identification of massive bleeding and protocolised approaches to the first rounds of resuscitation. Haemostatic blood products are available at the bedside immediately, and resuscitation starts with the use of equal proportions of platelets, plasma and RBCs. (If apheresis platelets are used, the proportions are 6 plasma : 6 RBCs : 1 apheresis platelet.) All products are warmed. (Six cold blood components will reduce the core temperature of a 70 kg individual by 1 °C and increase haemorrhagic mortality by 10%.) As resuscitation progresses and mechanical control of bleeding is achieved, additional rounds of component use are goal-directed based on cardiovascular response and algorithm-driven, focused laboratory assessments.

The Limits of Conventional Blood Products

Giving plasma and platelets to restore blood volume and support coagulation and RBCs to maintain oxygen transport are the central tenets of haemostatic resuscitation. However, this process does not reconstitute whole blood. The blood lost by the patient – and the unit of whole blood given by the donor – starts with a haematocrit of perhaps 40%, a platelet count of about 250×10^9/L and plasma coagulation factors equivalent to roughly 1 U/mL. That unit of donor whole blood is then diluted with 70 mL of citrate anticoagulant and 110 mL of RBC additive solution, and undergoes additional losses of cellular components and plasma in the leucocyte-reduction filters and processing steps.

When the components are added back together and given to the patient, the resulting haematocrit is around 29%, the platelet count about 140×10^9/L and the concentration of clotting factors about 0.65 U/mL. All compete for space in the blood volume, as

Box 30.3 Current principles of trauma resuscitation.

- There is an acute coagulopathy of trauma. Volume resuscitation with crystalloid will make it worse. Early administration of haemostatic blood components for volume resuscitation appears to improve outcome.
- Clinical judgement of massive haemorrhage risk is poor, and worse in massive maternal and paediatric haemorrhage. Assessment scores, such as the Assessment of Blood Consumption (ABC) score, improve prediction but are based on young adult males.
- The ABC score gives one point each for (1) systolic blood pressure < 90, (2) pulse > 110, (3) penetrating mechanism and (4) evidence of free intra-abdominal blood by focused abdominal sonography for trauma (FAST). Score ≥ 2 has a 50% risk of massive haemorrhage.
- Patients in deep shock must go straight to the operating room for attempted haemorrhage control. Those who can tolerate delay get computed tomography scanning to further define extent of injury and sites of bleeding.
- Catastrophically bleeding patients die fast. Proactive anticipation of a massive bleeding situation and initiation of a massive transfusion protocol can be life-saving. The protocol can always be cancelled if not needed.
- Starting resuscitation using blood components in a 1 : 1 : 1 unit ratio platelets : plasma : red blood cells (RBCs) ensures early coverage of all likely deficits. This requires early access to thawed universal donor plasma, type AB or type A with low-titre B. The proportion with apheresis platelets is 1 : 6 : 6.
- Resuscitation should be goal directed to correct laboratory-defined deficits as soon as possible. Emergency haemorrhage panels (haematocrit, platelet count, prothrombin time/international normalised ratio and fibrinogen) can be available quickly and guide administration of RBC, platelet, plasma and cryoprecipitate, respectively. Thromboelastography (TEG) or rotational thromboelastography (ROTEM) is less accurate and less directly correlated with blood component needs.

the plasma dilutes the RBCs and the additive solution in the RBCs dilutes the plasma. In addition, about 10% of the transfused RBCs and 30% of the transfused platelets are lost because of storage-related cellular injury that limits *in vivo* recovery. Finally, a third of platelets go to the spleen. The end result is that giving massively bleeding patients components in a 1 : 1 : 1 ratio of units of plasma, platelets and RBCs – even with no additional crystalloid or colloid – barely keeps the haematocrit above 25%, the platelet count above 50×10^9/L and the PT and PTT below 1.5 times normal. Altering the ratio in favour of any one product will dilute the other two. Giving crystalloid or colloid fluid dilutes all three. Transfusing 1 : 1 : 1 is an excellent way to 'fly blind', but transitioning as quickly as the bleeding rate allows to goal-directed therapy, whereby specific products are administered to correct specific deficits as dictated by laboratory testing, must be a focus in managing patients with massive haemorrhage.

Multicomponent resuscitation also leads to acidosis, hypothermia and citrate-driven hypocalcaemia. Laboratory tests of reconstituted blood in a 1 : 1 : 1 ratio found an average pH of 7.11, sodium of 150 mmol/L and potassium of 5.1 mmol/L, and no detectable ionised calcium [8]. Infusion of refrigerated and room-temperature blood leads to rapid hypothermia, which exacerbates cardiovascular instability and coagulopathy, so all fluids and blood products should be warmed [9,10]. The healthy liver can rapidly clear citrate, but, during severe bleeding, hypoperfusion of the liver and rapid infusion of blood products will

quickly lead to hypocalcaemia, exacerbating hypotension and perfusion. Infusion of up to 200–300 mg of calcium chloride per round of balanced resuscitation has been recommended to maintain normocalcaemia and blood pressure.

Donor whole blood is now available again in some large centres and in remote areas that have maintained whole blood donor programmes as their only recourse [11,12]. Whole blood is attractive for high-volume resuscitation because of its ease of use (1 bag rather than 3), higher concentrations of all components and ability to deliver haemostatic resuscitation in prehospital care. However, given that at present 50% of all blood product use is as components (mainly platelets) in cancer care, the paradigm shifts in blood collection and processing systems required to supply the relatively 'niche' market for whole blood is unlikely to happen soon. Knowing how to use blood components effectively and minimise their limiting factors will continue to be vital in the support of bleeding patients.

Laboratory Support

The ideal laboratory test for detecting the acute coagulopathy of trauma does not exist. Commonly used tests include conventional measures of coagulation – PT/INR, platelet count, fibrinogen – viscoelastic tests – thromboelastography (TEG®), rotational thromboelastography (ROTEM®) – and more specialised tests like the platelet function analyser PFA-100 and platelet aggregometry. Laboratory testing during acute bleeding is limited by the multifactorial nature of coagulation, the lag time between sampling and results, and the imperfect nature of all existing coagulation tests. Under the circumstances of massive haemorrhage, delays in laboratory reporting can be reduced to under 20 minutes with institutional commitment to procedural change. Other than in cost (conventional coagulation tests are considerably cheaper than viscoelastography), no study has been able to conclusively demonstrate the superiority of one method over the other. What is most important is that every institution caring for massively bleeding patients has in place comprehensive coagulation testing protocols within its massive transfusion protocols, and that local care teams are comfortable making decisions based on the results.

Evidence

The first empirical signs of the efficacy of haemostatic resuscitation approaches were decreasing rates of pulmonary and visceral oedema, decreasing intensive care unit (ICU) stays for ventilation and delays in abdominal closure. As clinical experience accumulated, mortality was reported as markedly lower in patients who received higher ratios of plasma to RBCs. Finally, overall population rates of haemorrhagic mortality and blood use declined.

From 2005 to 2009, first in civilian but also in military battlefield casualties, reports emerged in the literature documenting improved survival with balanced blood component resuscitation. Johansson and Stensballe at the University of Copenhagen described a 30% decrease in the mortality of massive transfusion associated with aortic rupture. Borgman and colleagues reported a 50% reduction in mortality in military casualties receiving plasma to RBCs at a 1 : 1 unit ratio, and the Dutch military described survivorship increasing from 44 to 84% in their military casualties after they shifted to 1 : 1 resuscitation in 2007. The seven US academic trauma centres involved in the Host Response to Injury Large Scale Collaborative Program noted a 50% decrease in the rate of patients who received 1 unit of RBCs, progressing to 10 units as the fraction of total plasma and platelets used in the first hours after admission increased. Cotton and colleagues reported improved survival and lower blood use in a large series of damage control laparotomies before and after the

institution of 1 : 1 : 1 resuscitation. The marked improvement in survival of grade V liver injury has been noted above.

To date, the Pragmatic Randomized Optimal Plasma and Platelet Ratios (PROPPR) trial is the only randomised trial of ratio-based transfusion [13]. The study was underpowered for the observed 30-day mortality of 24%, and the differences in mortality between 1 : 1 : 1 and 1 : 1 : 2 study arm ratios of plasma : platelets : RBCs were not different at the primary safety endpoints of 24 hours or 30 days. Among secondary endpoints, significantly more patients achieved anatomical haemostasis, and significantly more haemorrhaging patients were alive at 3 hours into resuscitation with 1 : 1 : 1-based therapy. At the end of the study, the absolute differences in haemorrhagic mortality between the arms remained, but equivalent numbers of patients died of central nervous system (CNS) injury and multiple organ failure. The case for haemostatic resuscitation remains strong, but is unsupported by level 1 evidence.

Can the Lessons of Damage Control Resuscitation for Trauma Be Extended to Other Massive Haemorrhage Situations?

As already noted, trauma accounts for only about 15% of deaths from massive haemorrhage. In whatever circumstances it occurs, massive bleeding generally appears to benefit from timely haemostatic intervention. However, protocols must be focused to respond to the unique haemodynamics and coagulopathies associated with each situation. Institutional massive transfusion protocols are needed for all the common situations of massive bleeding, overseen with scientific expertise and managed as critical quality-of-care resources. Better scientific understanding of these situations is very much needed; avenues for primary and secondary prevention of massive haemorrhage remain a goal.

Massive transfusions in pregnant patients and small children are important examples of the complexities of developing effective protocols in special populations. Life-threatening maternal haemorrhage often develops precipitously. This requires planned responses for the hospital, the birthing centre and the home. Although no specific coagulopathy of maternal haemorrhage is recognised, early support with fibrinogen may be important.

Massive transfusion in very small children is complicated by volume considerations: adult unit sizes are roughly equivalent to the entire blood volume of an infant. In small children, aliquots of about 10 mL/kg of RBCs and plasma can be alternated, converting the plasma to platelets in plasma (not in additive solution) as soon as possible, and staying alert to the physiological and haemodynamic distinctions of children less than 6 months old. Because this regime is complex and requires special equipment, a special paediatric massive transfusion protocol is important and requires frequent practice.

Tranexamic Acid

The prospective World Maternal Antifibrinolytic (WOMAN) and CRASH-II Trials provided level 1 evidence for the safety and efficacy of the use of tranexamic acid for uncontrolled bleeding associated with fibrinolysis if used within the first 3 hours of tissue injury (including surgical incisions or placental separation). Beyond that time, no benefit was seen.

Conclusion

The management of bleeding and coagulopathy in massive blood loss has been an area of major research for the last 15 years and continues to be so. The priority of initial treatment is to maintain perfusion while improving haemostasis, including surgical control, coagulation factor support and normothermia.

KEY POINTS

1) Massive haemorrhage is frequently accompanied by volume loss, haemodilution, hypothermia, acidosis, factor consumption and fibrinolysis. In trauma, the latter four issues are complicated by a trauma-associated coagulopathy.
2) Critically bleeding patients die fast. Effective response to massive bleeding is complex and requires tight, well-considered teamwork at all levels: clinical and laboratory.
3) Start resuscitation of severely injured patients with protocolised use of whole blood or equal proportions of plasma, red blood cells and whole blood donor platelets. Start with plasma or platelets. If apheresis platelets are used, the proportions are 6 plasma : 6 red blood cells : 1 platelets.
4) Use a blood warmer. Six blood components at 4 °C will reduce the core temperature of a 70 kg individual by 1 °C and increase the risk of haemorrhagic mortality by 10%.
5) Convert to goal-directed therapy based on cardiovascular response and focused laboratory assessments as soon as possible.
6) Essential laboratory response includes the ability to return results for haematocrit, platelet count, prothrombin time (PT)/international normalisation ratio (INR) and fibrinogen within 30 minutes. Order as an 'emergency haemorrhage panel' at least every 30 minutes during massive transfusion.
7) Massive obstetrical or paediatric haemorrhage are driven by the same physiological concerns, but each has unique aspects that must be considered and incorporated into protocols for those patients. Consider early fibrinogen support in maternal bleeding and plan carefully for the special volume needs of very small children.
8) At present, tranexamic acid is widely used as an adjunct to block fibrinolysis and to stabilise clot formation. Tranexamic acid appears to be safe and effective when used within 3 hours of tissue injury (including surgical incision or onset of uterine bleeding).

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31 Transfusion in Non-Bleeding Medical and Surgical Patients

Killian Donovan[1,3], *Akshay Shah*[2,3], *Stuart McKechnie*[3,4] *and Simon J. Stanworth*[2,5,6]

[1] *Oxford University Clinical Academic Graduate School, John Radcliffe Hospital, Oxford, UK*
[2] *Radcliffe Department of Medicine, University of Oxford, Oxford, UK*
[3] *Adult Intensive Care Unit, Oxford University Hospitals NHS Foundation Trust, Oxford, UK*
[4] *Nuffield Department of Anaesthesia, Oxford University Hospitals NHS Foundation Trust, Oxford, UK*
[5] *Transfusion Medicine, NHS Blood and Transplant, Oxford, UK*
[6] *Department of Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford, UK*

Transfusion of allogeneic blood components is a common intervention, with approximately 80 million donor-derived units transfused annually worldwide. Many of these transfusions are given to non-bleeding patients. These transfusions are therefore prophylactic: to prevent the complications of anaemia or to prevent bleeding. Unlike the situation of acute bleeding, in the case of a non-bleeding patient there is generally adequate time for a carefully considered decision surrounding the risks and benefits of transfusion strategies, or the use of alternatives.

Serious harm can be associated with the transfusion of blood components given they are biological agents, and there are general principles that can be applied to transfusion to reduce these harms. In the setting of prophylaxis these principles are more relevant. *Primum non nocere*.

First, the quantity or dose of blood component transfused should be the minimum necessary for a given evidence-based indication. The best transfusion may often be no transfusion at all.

Secondly, consideration should be given to alternatives to transfusion. For example, in the case of patients who are able to tolerate anaemia, iron supplementation or recombinant human erythropoietin (rhEPO) may be appropriate. This practice is already embedded in certain clinical areas, notably renal and perioperative medicine, but remains to be broadly implemented among the population of acute general medical or surgical patients. In the field of perioperative medicine this concept is now framed as patient blood management, the principles of which are (i) minimising blood loss and bleeding, (ii) optimising haematopoiesis and (iii) optimising adaptation to anaemia while instituting appropriate treatment.

Many randomised trials and meta-analyses have addressed questions surrounding transfusion in non-bleeding patients, including transfusion thresholds, product selection and harms. Although the optimal transfusion triggers remain undetermined in many clinical situations, there is little evidence to suggest that transfusion to the population normal range of haemoglobin (Hb), platelet

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count or laboratory coagulation parameters confers benefit relative to more restrictive transfusion strategies. The following sections will survey the evidence surrounding transfusion of specific products in the non-bleeding patient and provide case vignettes to illustrate application of this evidence.

Red Cell Transfusion

The goal of red cell transfusion is to restore the oxygen carrying capacity of blood and to improve tissue oxygen delivery, both of which may be impaired by anaemia. In stable patients, anaemia may manifest as non-specific symptoms of impaired exercise tolerance, breathlessness or fatigue. In the context of critical illness, particularly patients with shock, tissue oxygen delivery ($DO_2 \approx CO \times SpO_2 \times [Hb]$) may be significantly impaired. However, in practice the assessment of systemic and organ-specific tissue oxygen delivery is difficult, with the clinical signs of shock and surrogate markers of global tissue oxygen delivery such as lactate concentration and mixed (or central) venous saturations lacking specificity. There are clinical situations such as acute coronary syndrome or traumatic brain injury where organ-specific (rather than global) oxygen delivery is a paramount concern. Because tissue oxygen consumption and delivery are difficult to measure directly, the most commonly used clinical parameter for deciding on red cell transfusion is the blood haemoglobin concentration. Other markers or clinical signs are occasionally employed to help with transfusion decisions, but international guidelines recommend against their use.

Thresholds for Red Cell Transfusion

The threshold haemoglobin concentration below which red cell transfusion is indicated continues to be debated. The value below which tissue oxygenation becomes impaired clearly exhibits great heterogeneity, with healthy individuals tolerating haemoglobin concentrations of around 4 g/dL with minimal symptoms, whereas older adults may become symptomatic at levels of 7–8 g/dL. Regardless of these interindividual differences, it is clear that satisfactory tissue oxygenation can be maintained with haemoglobin concentrations significantly below the normal range. This appears to be the case even in critically ill patients, where tissue oxygen consumption may be significantly elevated. Randomised trials of packages of 'goal-directed therapy' including transfusion to near normal range of haemoglobin in the critically ill have consistently failed to demonstrate improvement in clinically important outcomes [1].

Arguments in favour of a more restrictive approach (for example a threshold of 7 g/dL) to red cell transfusion have focused on ethical issues of resource utilisation and on the potential harms of overtransfusion. In addition to the well-characterised immunological transfusion reactions and risks of transfusion-associated infection, observational studies suggest that patients receiving allogeneic red cells have an increased risk of death, delayed wound healing and increased length of stay, although these studies may have residual confounding by indication [2].

There is strong evidence supporting the safety and non-inferiority of restrictive transfusion strategies, with a multitude of randomised trials across various fields and a 2016 meta-analysis incorporating 31 randomised controlled trials including 12 587 participants [3]. The clinical scenarios in which the safety of restrictive transfusion has been demonstrated in randomised trials include critical illness, perioperative care, septic shock and gastrointestinal bleeding.

Red Cell Transfusion in Critical Illness (see Box 31.1)

The Transfusion Requirements in Critical Care (TRICC) trial [4] in the late 1990s was

Box 31.1 Case vignette.

A 69-year-old male with a background of coronary artery disease and stable angina has been admitted to the intensive care unit (ICU) with septic shock secondary to pyelonephritis. He has a minimal oxygen requirement (FiO_2 30%), and is requiring high doses of noradrenaline to maintain a mean arterial pressure of 65 mmHg. His urine output is adequate, and his blood lactate concentration is 4 mmol/L. His haemoglobin concentration is 7.4 g/dL. There is no clinical evidence of bleeding. Is a red cell transfusion indicated?

This typical case demonstrates some of the practical transfusion challenges in critical care. This gentleman has shock with evidence of globally impaired tissue oxygen delivery (lactataemia). There are additional concerns regarding tissue-specific myocardial oxygen delivery, given his background of coronary artery disease. While evidence from randomised studies clearly demonstrates the safety of restrictive transfusion thresholds (7 g/dL) in septic shock (TRISS trial), should the presence of coronary disease change our threshold in this case?

There is a theoretical concern that the myocardium may be more vulnerable to anaemia than other tissues, as the baseline oxygen extraction is near maximal (≈50–60%) in this capillary bed. Meta-analysis including trials from various clinical settings demonstrates an association between restrictive transfusion and increased risk of myocardial infarction in patients with coronary artery disease. National guidance (British Society of Haematology [13]) recommends a threshold of 7 g/dL for those with stable angina (grade 2B) or 8–9 g/dL for those with acute coronary syndrome (grade 2C). In this particular case, a reasonable evidence-based case could be made for keeping a threshold of 7 g/dL in the absence of chest pain or dynamic electrocardiogram (ECG) changes, in which case a higher threshold may be appropriate. European Society of Intensive Care Medicine guidance suggests a threshold of 9 g/dL in the context of an acute coronary syndrome [7].

$$DO_2 = CO \times \{(1.34 \times [Hb] \times sO_2) + (K \times P_aO_2)\}$$

DO_2 : Oxygen delivery in ml/min
CO : Cardiac output in L/min
[Hb] : Blood haemoglobin concentration
sO_2 : Haemoglobin saturation
K : plasma solubility constant for oxygen
P_aO_2 : arterial partial pressure of oxygen

Figure 31.1 The oxygen delivery equation.

conducted in an era when transfusion to a haemoglobin of 10 g/dL was common practice in critical care due to a desire to maximise tissue oxygen delivery (DO_2) (Figure 31.1) and concerns from observational studies that anaemia was associated with higher mortality. This trial randomised 838 critically ill, non-bleeding participants to a 'restrictive' transfusion strategy (transfusion trigger of 7 g/dL with a target haemoglobin between 7 and 9 g/dL) or a 'liberal' transfusion strategy (transfusion trigger of 10 g/dL with a target range of 10–12 g/dL). The study demonstrated a 50% reduction in red cell transfusion in the restrictive group, with no overall difference in 30-day mortality. Myocardial infarction and pulmonary oedema were more common in the liberally transfused group and other adverse events were equal. Prespecified subgroup analyses suggested that restrictive transfusion may be superior to a liberal strategy among patients with lower illness severity scores (APACHE II score < 21) and among patients under 55 years of age.

Particular uncertainty persisted regarding transfusion in critically ill patients with shock. Based on physiological rationale, it was presumed that transfusion to a higher haemoglobin would augment tissue oxygen

delivery (Figure 31.1) and ameliorate the organ dysfunction associated with shock. For many years, a liberal haemoglobin target was incorporated into goal-directed resuscitation strategies [5]. The [Hb] component of this strategy was tested in septic shock in the TRISS trial [6]. This trial randomised 1005 patients with septic shock to receive red blood cell transfusions when the haemoglobin dropped below 7 g/dL (restrictive) or 9 g/dL (liberal). Patients in the restrictive group received approximately 50% fewer red cell transfusions and had similar 30-day survival and requirements for organ support, which suggested that a restrictive transfusion is safe in septic shock.

The European Society of Intensive Care Medicine (ESICM) guidelines now advise that for non-bleeding patients in the general intensive care unit (ICU), the threshold for red blood cell transfusion should be 7 g/dL [7] (strong recommendation, moderate certainty), and the use of red cell transfusions in ICU has declined over recent years (Table 31.1).

Perioperative Anaemia

In the setting of elective surgery, preoperative anaemia is common and is associated with longer hospital stay, higher in-hospital mortality and increased risk of acute myocardial infarction and stroke [8]. In many settings there is an opportunity to identify and treat anaemia preoperatively. As such, there should rarely be a need for red cell transfusion to facilitate elective surgery. Randomised trials have investigated the role of iron infusion to treat preoperative anaemia, with the recent PREVENTT trial demonstrating that intravenous (IV) iron successfully increased haemoglobin, but had no effect on transfusion requirement or perioperative mortality [9].

Postoperative anaemia is more common due to blood loss, increased red cell turnover and haemodilution. In keeping with the concept of patient blood management, this can be minimised by using adjuncts such as cell salvage and tranexamic acid where indicated. In the event that postoperative anaemia does occur, randomised evidence can guide decisions surrounding transfusion. Carson and colleagues randomised 2016 patients with anaemia following hip surgery to receive allogeneic blood transfusion at a threshold of 8 g/dL (restrictive) or 10 g/dL (liberal) [10]. In this population there was a substantial reduction in the number of red cell units transfused and no difference in mortality or functional outcome at 60 days. The TRICS-III trial [11] randomised over 5000 patients undergoing cardiac surgery to a transfusion threshold of 7.5 g/dL (restrictive) or 8.5 g/dL (liberal), with a primary outcome of death, myocardial infarction, stroke or renal failure by day 28. There was no difference in the composite outcome between the groups and a substantial reduction in the amount of blood transfused.

Although there is variation in the thresholds used in various trials, the overall trend is apparent: restrictive transfusion thresholds are safe in the perioperative period. The Frankfurt Consensus Conference on Patient Blood Management in 2018 made recommendations of thresholds of 7 g/dL for the stable postoperative ICU patient (strong recommendation), 8 g/dL for hip fracture patients (conditional recommendation) and 7.5 g/dL for patients following cardiac surgery (strong recommendation) [8].

Acute Coronary Syndrome

Myocardial infarction is caused by an acute mismatch between myocardial oxygen delivery and demand. As the myocardial extraction ratio is near maximal at baseline, there is a theoretical rationale that augmenting the haemoglobin concentration and oxygen carrying capacity of the blood may improve myocardial oxygen delivery and thus reduce ischaemia.

A systematic review and meta-analysis [12] of trials comparing transfusion thresholds among patients with cardiovascular disease or acute myocardial infarction demonstrated no mortality difference between transfusion

Table 31.1 Sample practical transfusion strategies.

Clinical scenario	Suggested strategy	Guidelines	Strength of recommendation and quality of evidence	Comments
Anaemia in a non-bleeding critically ill patient	Address reversible causes of anaemia (haematinic deficiencies, haemolysis etc.) Transfusion of red cells if haemoglobin < 70 g/L	ESICM 2020 [7]	Strong recommendation, moderate certainty	
Anaemia in a critically ill patient with acute coronary syndrome	Address reversible causes of anaemia Transfusion of red cells if haemoglobin < 90–100 g/L	ESICM 2020 [7]; BSH 2012 [13]	Conditional recommendation, low certainty	ESICM recommends a threshold of 90–100 g/L; BSH recommends 80–90 g/L
Elevated prothrombin time in a critically ill non-bleeding patient	Reverse anticoagulants if indicated. Administer vitamin K if suspicion of deficiency Do not prophylactically transfuse FFP	ESICM 2020 [7]	Conditional recommendation, very low certainty	The benefits of FFP transfusion even in patients undergoing invasive procedures are uncertain. It is not clear that FFP transfusion either corrects laboratory parameters or reduces periprocedural bleeding risk
Elevated prothrombin time in a non-bleeding patient undergoing high-risk invasive procedures	Reverse anticoagulants if indicated. Administer vitamin K if suspicion of deficiency Transfuse FFP. Target INR should be < 1.5–1.8	Society of Interventional Radiology 2019 [20]	Weak recommendation, level D evidence	
Elevated prothrombin time in a non-bleeding patient with cirrhosis undergoing paracentesis	Do not routinely measure laboratory measures of coagulation prior to paracentesis	BSG 2021 [22]	Strong recommendation, moderate quality of evidence	
Thrombocytopaenia in a non-bleeding critically ill patient	Transfuse platelets if count < 10×10^9/L A threshold of 50×10^9/L may be appropriate in invasive procedures including tracheostomy or CVC insertion	ESICM 2020 [7]	Conditional recommendation, very low certainty	Previous guidance recommended a higher threshold of 20×10^9/L for patients with sepsis based on the probability that the platelet turnover in these patients is higher

BGS, British Society of Gastroenterology; BSH, British Society of Haematology; CVC, central venous catheter; ESICM, European Society of Intensive Care Medicine; FFP, fresh frozen plasma; INR, international normalised ratio.

strategies, but an increased risk of new myocardial infarction among those patients allocated to a restrictive strategy (risk ratio [RR] 1.78, 95% confidence interval [CI] 1.18–2.70). The ESICM recommends a liberal transfusion threshold of 9 g/dL in patients with acute coronary syndromes (conditional recommendation, low certainty) [7,13]. A very recent trial randomised 668 patients with acute myocardial infarction, comparing restrictive versus liberal transfusion strategies (haemoglobin ≤ 8 g/dL or ≤ 10 g/dL), and reported no differences in the composite outcome of all-cause death, stroke, recurrent myocardial infarction or emergency revascularisation at 30 days, but the study may have been underpowered to identify clinically important differences [14].

Neurological Injury

In brain or spinal cord injury there is little evidence to guide allogeneic red cell transfusion. The cerebral microcirculation is physiologically distinct from other organ systems and as a result it is speculated that the injured brain and penumbra are more vulnerable than other tissues to hypoxic damage. Small, randomised trials in patients with head injury and subarachnoid haemorrhage have not however demonstrated the superiority of any particular transfusion strategy. A larger trial in 200 head-injured patients investigating the use of EPO, while also randomising patients to receive allogeneic red cells with a threshold of 7 g/dL versus 10 g/dL, demonstrated no difference in disability at six months, but had a higher rate of venous thromboembolism in those randomised to a higher haemoglobin threshold, although this may be related to rhEPO use rather than transfusion *per se* [15].

Larger randomised trials are in progress (NCT02968654 and NCT03309579), and alternative approaches to understanding the metabolic environment of the injured brain, including advanced imaging and cerebral microdialysis, are needed and may help to guide future practice in this field.

Fresh Frozen Plasma

Fresh frozen plasma (FFP) is usually administered in the non-bleeding patient prophylactically to prevent bleeding. In a UK audit in 2009, 43% of FFP units (or concentrates) were administered to patients with no documented evidence of bleeding. Transfusion of FFP in this context is usually guided by abnormal laboratory coagulation tests and a perception of elevated bleeding risk. High-quality evidence to support this practice is lacking, and clinical and *in vitro*/*in vivo* data have demonstrated that laboratory measures of coagulation do not correlate with periprocedural bleeding risk in most clinical contexts. Furthermore, transfusion of FFP does not reliably correct mild derangements of laboratory coagulation parameters [16]. We consider here the evidence and guidelines surrounding use of prophylactic FFP transfusions.

Fresh Frozen Plasma for Bleeding Prophylaxis

The activated partial thromboplastin time (aPTT) assay was developed to investigate hereditary haemophilia. The historical development of the prothrombin time (PT) assay is linked to the development of warfarin and related anticoagulants. Outside of these contexts, systematic reviews of observational studies have demonstrated that these standard laboratory measures of coagulation do not predict bleeding associated with invasive procedures [17]. Yet correction of coagulopathy (elevated PT or aPTT) remains one of the most frequent uses for FFP in clinical practice.

Coagulopathy is common in critically ill patients, affecting approximately 30% of patients in the ICU. These patients are also routinely exposed to invasive procedures. Despite abnormalities of standard laboratory tests, these patients often have intact thrombin generation, and this is not affected by FFP administration [18]. In a randomised trial in the ICU that allocated critically ill

patients with coagulopathy (international normalised ratio [INR] > 1.5) about to undergo invasive procedures to receive 12 mL/kg FFP or no FFP, there was no difference in bleeding outcomes between the groups [19], and thromboelastographic measures of clot formation were not altered by FFP administration.

Special consideration is often given to high-risk bleeding procedures such as neuraxial anaesthesia and liver biopsy, but there is little clinical evidence to guide practice in these areas. The Society of Interventional Radiology Standards of Practice Committee in 2019 acknowledged the low standard of available evidence and recommended against routinely checking coagulation parameters for patients with no risk factors for bleeding undergoing low-risk procedures, suggesting that most low-risk procedures could safely be performed with an INR in the range 2–3. For higher-risk procedures it recommended correction to less than 1.5–1.8 [20]; however, the available laboratory and clinical evidence suggests that correction of mild derangements of laboratory coagulation measures is not successfully achieved with FFP transfusion [16].

Fresh Frozen Plasma in Patients with Cirrhosis (see Box 31.2)

Coagulopathy in cirrhosis is considered distinct from other causes, as patients are often observed to be hypercoagulable despite a significantly elevated INR. This concept of 'rebalanced haemostasis' reflects concomitant changes in both pro- and anticoagulant pathways. Both bleeding and thrombotic complications are seen in these patients, who require many invasive procedures, including paracentesis and liver biopsy.

Due to these limitations of standard laboratory measures, research has shifted towards alternative methods of identifying patients at high risk of bleeding. A randomised trial sought to identify a role for thromboelastography in guiding transfusion in cirrhotic patients undergoing invasive procedures. Such a strategy reduced the use of FFP and demonstrated no increase in bleeding rates. There was only a 2% bleeding

Box 31.2 Case vignette.

A 49-year-old male with decompensated cirrhosis due to alcoholic liver disease presents with tense ascites and requires paracentesis. His prothrombin time (PT) is 22.3 seconds, his activated partial thromboplastin time (aPTT) is 40 seconds and his platelet count is 97 × 10^9/L. Are blood products necessary for safe paracentesis?

Bleeding risk around the time of a procedure depends upon both patient factors and procedural factors. In general, abdominal paracentesis is considered a low-bleeding-risk procedure. How does the presence of coagulopathy and thrombocytopaenia alter the risk?

The coagulopathy of liver disease is often termed 'rebalanced haemostasis', as these patients typically exhibit a tendency to thrombosis rather than bleeding, even in the context of deranged laboratory indices of clotting. Observational studies have shown that the risk of bleeding following paracentesis is not predicted by preprocedural prothrombin time or platelet count, and recent guidance from the British Gastroenterology Society [22] recommends against measuring these parameters routinely before paracentesis, and against infusion of blood products prior to paracentesis (strong recommendations, moderate quality of evidence). Evidence for higher-risk bleeding procedures such as biopsies of viscera is lacking.

Observational data suggest that coagulopathy is common in critical illness, but the use of fresh frozen plasma (FFP) does not correct this. Thromboelastography or novel methods of measuring thrombin generation may provide a better estimate of *in vivo* clot formation, but how to apply these techniques in this context remains unclear.

rate overall, even though all participants were coagulopathic by standard laboratory coagulation tests [21]. Despite the lack of high-quality evidence, it is common practice to use periprocedural FFP in cirrhotic patients with deranged laboratory measures of coagulation for high-bleeding-risk procedures. National guidance in the UK [22] now recommends against the use of blood products for therapeutic paracentesis (Table 31.1).

Conclusion

FFP transfusion is associated with risks including circulatory overload, transfusion-related acute lung injury (TRALI) and transfusion-transmitted infection. There is little evidence for its effectiveness either in correcting abnormal tests of coagulation, or in reducing bleeding risk in the non-bleeding patient across a range of clinical scenarios. A key step towards improving transfusion practice in this area will be the development of better diagnostic tests that can more accurately identify those patients at risk of bleeding.

Platelet Transfusion

In non-bleeding patients, platelets are usually administered to prevent bleeding in those who are thrombocytopaenic, or to ameliorate the bleeding tendency associated with antiplatelet medications prior to an invasive procedure.

In the case of thrombocytopaenia, a threshold value of platelet count is generally considered to be the trigger for transfusion, but this does not provide any information on platelet function. Evidence from randomised trials of platelet thresholds is largely from patients with haematological malignancy and neonates with thrombocytopaenia [23]. These trials have demonstrated the safety of restrictive thresholds for platelet transfusion (10×10^9/L among adults with haematological malignancy). In the absence of high-quality evidence in other clinical scenarios, this evidence has been extrapolated to other contexts.

Use of Platelets in the Critically Ill (see Box 31.3)

Thrombocytopaenia is frequent in critical illness due to increased platelet turnover and myelosuppression. Between 8% and 66% of ICU patients are thrombocytopaenic. Approximately 9% of these patients receive platelet transfusions [24]. Despite this, there is no evidence from randomised trials to guide the use of platelet transfusions in the critically ill. The ESICM guidelines extrapolate directly from trials in patients with haematological malignancy and advise platelet transfusion if the platelet count falls below 10×10^9/L (conditional recommendation, very low certainty) [7].

Use of Platelets for Procedural Prophylaxis

Higher platelet thresholds are often utilised for patients undergoing invasive procedures. There is wide variation in practice concerning thresholds for platelet transfusion [25]. The ESICM recommends against platelet transfusions for periprocedural prophylaxis for central venous catheter (CVC) insertion or percutaneous tracheostomy at a level of 50×10^9/L or above (conditional recommendation, very low certainty), but makes no recommendation below this level [7]. International interventional radiology guidelines recommend considering transfusion of platelets for high-risk procedures if the platelet count is below 50×10^9/L (weak recommendation, level D evidence) and for all procedures if the count is below 20×10^9/L (weak recommendation, level D evidence) [20]. A Cochrane review investigating the role of prophylactic platelet transfusion prior to surgery found insufficient evidence to make a recommendation [26]. The generally accepted threshold platelet count for major abdominal surgery is 50×10^9/L, but there is little evidence to

Box 31.3 Case vignette.

A 21-year-old female is admitted to the intensive care unit (ICU) following a car accident where she sustained severe burns. She has developed recurrent episodes of sepsis and associated coagulopathy and thrombocytopaenia. She is currently intubated and mechanically ventilated, dependent on two vasopressors and undergoing continuous renal replacement therapy. There is no evidence of bleeding. Her platelet count is 15 × 10^9/L. Is transfusion indicated?

The decision to transfuse platelets is based upon the platelet count, assessment of the bleeding risk and the aetiology of the thrombocytopaenia. The risk of spontaneous bleeding at a platelet count of 15 × 10^9/L is generally not thought to be substantially elevated, and the European Society of Intensive Care Medicine [7] recommends against prophylactic transfusions unless the platelet count falls below 10 × 10^9/L. In this patient's case, it is also unlikely that transfusion will result in a substantial increment in the platelet count, as the cause of the thrombocytopaenia is likely excessive consumption in the setting of multiorgan failure and sepsis.

However, this patient will require vascular procedures in her ICU stay for line replacements and surgery for her burns. Consensus guidelines suggest administering platelets to patients undergoing placement of non-tunnelled central venous catheters with a platelet count < 50 × 10^9/L. A practical approach in this case would therefore be to try to arrange invasive procedures for the same day and transfuse as appropriate prior to these procedures, in order to avoid separate episodes of transfusion for individual procedures.

support this. In the case of surgery in critical sites (intracranial, spinal or ocular), there is expert consensus that a threshold of 100 × 10^9/L be used. Understanding how to use platelets as prophylaxis is an area in pressing need of research.

Harm from Excessive Use of Platelets

Observational data indicate that platelet transfusions are used more liberally in clinical practice than is recommended by guidelines. Evidence from at least two randomised trials suggests that platelet transfusions in inappropriate clinical contexts may cause excess bleeding and death. The PlaNet-2 trial [27] was a randomised trial in neonates with thrombocytopaenia (less than 50 × 10^9/L) in which 600 participants were allocated to receive prophylactic platelets at a threshold level of 25 × 10^9/L or 50 × 10^9/L. Neonates randomised to the higher threshold group were more likely to receive a platelet transfusion (hazard ratio 2.75; 95% CI: 2.36–3.11) and had a higher rate of major bleeding or death compared with those in the lower threshold group. The PATCH trial [28] randomised 190 patients with intracerebral haemorrhage and concurrent antiplatelet medication use to receive platelets or no platelets. Participants who received platelets had a higher rate of death or disability at three months compared with those who did not. Although this result had been questioned in some secondary analyses due to baseline imbalances, when taken together with PlaNet-2, the results suggest that the expected benefit of platelet transfusions may not exist, and the harms may be greater than clinicians often perceive. Understanding how platelet transfusions may cause harm, possibly mediated through inflammatory pathways, is also a topic of research priority.

KEY POINTS

1) Transfusion to the normal range of haemoglobin, platelet count or coagulation parameters is unlikely to be clinically beneficial and is seldom indicated.
2) A restrictive haemoglobin transfusion threshold of 7 g/dL for red cell transfusion in the non-bleeding patient is safe in most clinical circumstances.
3) Fresh frozen plasma (FFP) transfusions are not indicated in most non-bleeding situations, irrespective of the level of abnormality of standard coagulation tests.
4) While platelets are frequently transfused to manage perceived bleeding risk in patients with platelet counts < 50×10^9/L, clinicians should recognise that the association between severity of thrombocytopenia and bleeding risk is not well established, and that evidence from randomised trials indicates harm from more liberal use of platelets.
5) Further research is required into better tests for identifying impaired oxygen delivery and predicting bleeding, and optimal thresholds for prophylactic transfusion of FFP and/or platelets.

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32 Transfusion in Malignant Haematological Disease

Lise J. Estcourt[1,3] *and Michael F. Murphy*[1,2,3]

[1] *NHS Blood and Transplant, John Radcliffe Hospital, Oxford, UK*
[2] *Department of Haematology, Oxford University Hospitals, Oxford, UK*
[3] *Radcliffe Department of Medicine, University of Oxford, Oxford, UK*

Patients with haematological diseases are major users of blood components. Haematological diseases requiring transfusion support cover a whole spectrum of clinical disorders: haemophilia (Chapter 28), benign haematological conditions (Chapter 33), haemoglobinopathies (Chapter 34), fetal, neonatal and paediatric practice (Chapter 35) and immune disorders (Chapter 36), in addition to haematological malignancies, which are the subject of this chapter.

The haemopoietic system has a dramatic capacity for increasing the production of blood cells, but this capability varies between different diseases. The scenario of anaemia related to marrow ablation following chemotherapy is very different from anaemia in an individual with a well-compensated chronic haemolytic process. Although 18% of all red cell units are transfused to patients with haematological disease, most are given to patients with malignant disorders [1]. The requirement for blood transfusions in this group is related to both the underlying condition itself and the myelosuppressive/myeloablative effects of the specific treatments used.

This chapter considers the following topics:

- The *indications* for red cell, platelet and granulocyte transfusions in patients with malignant haematological conditions.
- The approaches to the management and prevention of *complications* associated with transfusions in patients with malignant haematological conditions, including the use of special types of blood components.

Red Cell Transfusions

The ready availability of red cell components means that anaemia can be easily treated. There are some special considerations in the management of anaemia that are applicable to haematology patients, as well as to other clinical groups:

- The cause should be established and treatment other than blood transfusion should be used where appropriate, for instance in patients with iron deficiency or

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megaloblastic or autoimmune haemolytic anaemia (AIHA; Chapter 33). Anaemia of malignancy may be due to the effects of marrow infiltration or therapy, 'inhibitory' cytokine-mediated influences leading to secondary anaemias (of chronic disorders) or low levels of erythropoietin.

- There is no universal 'trigger' for red cell transfusion, i.e. a given level of haemoglobin concentration (Hb) at which red cell transfusion is appropriate for all patients. Clinical judgement balancing factors such as quality-of-life indices plays an important role in the decision to transfuse red cells or not [2].

Patients Receiving Intensive Myelosuppressive/ Myeloablative Treatment

There are specific considerations relating to the use of red cell transfusion in patients receiving intensive myelosuppressive/myeloablative treatment, including the need to provide a 'reserve' in case of severe infection or haemorrhage, and the convenience of having a standard policy for red cell transfusion in the setting of an acute haematology service.

The level of Hb used as the 'trigger' for transfusion varies from centre to centre, but is usually in the range of 70–90 g/L. A restrictive policy is generally advocated because of data from trials in other patient groups indicating the safety of this approach and the well-recognised risks of transfusion [2]. There are no definite data to support the use of a higher level, although studies in animal models of thrombocytopenia and in uraemic patients suggest that correction of anaemia also results in correction of prolonged bleeding times [3]. Furthermore, in a recent trial of patients undergoing haemopoietic stem cell transplantation, the use of a restrictive red cell transfusion strategy threshold of 70 g/L was as effective as a threshold of 90 g/L and resulted in similar quality of life and transplant outcomes with fewer transfusions [4].

Red Cell Transfusions and Chronic Anaemias

In patients with chronic anaemia requiring regular transfusions, red cell transfusions should be used to maintain the Hb just above the lowest level not associated with symptoms of anaemia. There is considerable variation in this level depending on the patient's age, level of activity and co-existing medical problems, such as cardiovascular and respiratory disease; for example, some young patients are asymptomatic with an Hb below 70 g/L, while some elderly patients are symptomatic even at an Hb above 100 g/L. Special considerations apply to patients with haemoglobinopathies, and these are considered in Chapter 34.

Use of Recombinant Erythropoietin in Haematological Disease

The clinical use of recombinant erythropoietin (RhEpo) might be considered in several situations in haematology patients, such as delayed erythroid engraftment after allogeneic bone marrow/peripheral blood progenitor cell transplantation, the treatment of anaemia in patients with myelodysplasia, and in the management of Jehovah's Witnesses with haematological disorders. Evidence supports an association between increases in Hb, reduced red cell transfusion requirements and possibly improvement in quality-of-life indices with RhEpo therapy, although the findings concerning quality-of-life measures are difficult to compare between studies. Uncertainties also remain about the factors predicting responsiveness, since a number of individuals fail to show adequate responses to RhEpo. There are also concerns about adverse events, especially thrombosis, in patients treated with RhEpo [5,6]. The most recent American guidelines [6] only recommend using RhEpo in patients with haematological malignancies who are being

treated with palliative intent, with the exception of selected low-risk patients with myelodysplastic syndromes.

Platelet Transfusions

In general, platelet transfusions are indicated for the prevention and treatment of haemorrhage in patients with thrombocytopenia or platelet function defects. The cause of the thrombocytopenia should be established before platelet transfusions are used, because they are not always appropriate treatment for thrombocytopenic patients, and in some instances are contraindicated, such as in thrombotic thrombocytopenic purpura, haemolytic–uraemic syndrome and heparin-induced thrombocytopenia (Chapter 16).

Indications for Platelet Transfusions

Therapeutic platelet transfusions are established as an effective treatment for patients who are bleeding. However, *prophylactic* platelet transfusion therapy for the prevention of haemorrhage in chronically thrombocytopenic patients with bone marrow failure associated with treatment for haematological malignancies remains controversial. Guidelines for platelet transfusion in many countries recommend that the platelet transfusion trigger for prophylaxis is 10×10^9/L [7], and most hospitals follow this recommendation, with an acceptance that selected patients with additional risk factors for bleeding, such as sepsis, might benefit from a higher threshold, such as 20×10^9/L. Unfortunately, many audits continue to document that compliance with these general recommendations is poor, and a recent national comparative audit in the UK [8] showed that a large proportion (25%) of prophylactic platelet transfusions were given inappropriately.

Another important issue for the use of prophylactic platelet transfusions is the dose of platelets. A large randomised controlled trial [9] showed there was no significant difference in the number of patients who bled between the low-dose (1.1×10^{11} platelets/m^2), medium-dose (2.2×10^{11} platelets/m^2) and high-dose (4.4×10^{11}/m^2) treatment arms. Overall, a low-dose transfusion policy reduced patients' total platelet requirements, but at the expense of a higher number of platelet transfusions. In the UK, the standard adult dose is approximately 2.4×10^{11} platelets, which is close to the low dose used in this trial. Interestingly, there was no difference in the number of platelet transfusion episodes between medium- and high-dose treatment arms, indicating that a high-dose policy decreases neither bleeding nor number of transfusion episodes.

A general finding across all prophylactic platelet transfusion trials has been the lack of any difference in results for haemostatic outcomes between trial arms (i.e. no increased bleeding in the restrictive policy arms for transfusion by lower threshold or dose). This has raised questions about the benefit of using platelet transfusions as prophylaxis to prevent bleeding. However, two randomised controlled trials of prophylactic platelet transfusions in adults with thrombocytopenia due to haematological malignancies both found that a no-prophylaxis approach led to higher rates of World Health Organization (WHO) grade 2–4 bleeding overall [10,11]. Platelet usage was markedly reduced in the no-prophylaxis arm in both studies. Prespecified subgroup analyses showed very similar proportions of bleeding between the prophylaxis and no-prophylaxis treatment arms in autologous haemopoietic stem cell transplant patients, in contrast to patients treated with chemotherapy or allogeneic haemopoietic stem cell transplant, raising the possibility of different platelet transfusion strategies depending on the patient's underlying condition and treatment [12].

A strategy of transfusing platelets only to treat bleeding is recommended for patients with chronic persisting thrombocytopenia due to bone marrow failure syndromes, such as myelodysplasia [7].

Prophylactic platelet transfusions for invasive procedures depend on the type of procedure being performed [7]:

- No increase in platelet count is required for low-risk procedures: bone marrow aspiration and biopsy, central line insertion.
- Consider performing the following procedures above the platelet count threshold indicated:
  - Venous central lines (both tunnelled and untunnelled), inserted by experienced staff using ultrasound guidance techniques, when the platelet count is $> 20 \times 10^9$/L.
  - Lumbar puncture when the platelet count is $\geq 40 \times 10^9$/L.
  - Insertion/removal of epidural catheter when the platelet count is $\geq 80 \times 10^9$/L.
  - Major surgery when the platelet count is $> 50 \times 10^9$/L.
  - Neurosurgery or ophthalmic surgery involving the posterior segment of the eye when the platelet count is $> 100 \times 10^9$/L.
  - Percutaneous liver biopsy when the platelet count is $> 50 \times 10^9$/L; consider transjugular biopsy if the platelet count is below this level.
- Prior to renal biopsy, ensure that potential risk factors for bleeding are corrected: anaemia (iron and erythropoietin), uraemia (dialysis); if renal biopsy is urgent consider desmopressin (DDAVP) pre procedure or oestrogen if time allows.

Granulocyte Transfusions

Severe persisting neutropenia is the principal limiting factor in the use of intensive treatment of patients with haematological malignancies. It may last for two weeks or more after chemotherapy or stem cell transplantation, and during this period the patient is at risk of life-threatening bacterial and fungal infections. The use of haemopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF), may reduce the duration and severity of severe neutropenia, but they are only effective if the patient has sufficient numbers of haemopoietic precursors. Moreover, the time to response may be several days. Supportive treatment with granulocyte transfusions is a logical approach, although a number of factors have limited its application:

- Difficulties in the collection of neutrophils, which are present in low numbers in normal individuals and are difficult to separate from red cells because of their similar densities (commercially available long-chain starch solutions now facilitate this separation).
- The short half-life of neutrophils after transfusion, coupled with short storage times and negative effects on function of prolonged storage.
- The frequent occurrence of adverse effects such as febrile reactions, including occasional severe pulmonary reactions and human leucocyte antigen (HLA) alloimmunisation causing platelet refractoriness.

Various methods have been used in the past to increase the number of neutrophils collected for transfusion, including obtaining granulocytes from patients with chronic myeloid leukaemia, treating donors with steroids and using hydroxyethyl starch to promote sedimentation of red cells. There was a further resurgence of interest in granulocyte transfusions, as larger doses of granulocytes could be collected from donors using regimens including G-CSF administered 12–16 hours prior to apheresis, together with oral steroids such as dexamethasone to further improve the yields.

Randomised controlled trials of granulocyte transfusions have been very challenging to undertake and complete. Recruitment to the Resolving Infections in Neutropenia with Granulocytes (RING) trial did not reach the required target [13]. There was no difference in primary endpoint (a composite 42-day outcome of survival and resolution of infection). It should also be noted that in this trial just under a third of patients received lower doses than defined in the protocol. Some groups

have suggested a potential role for an 'off-the-shelf' component of granulocytes derived from whole blood donations, although the doses of neutrophils are lower [14].

High-dose granulocyte transfusions collected from donors treated with G-CSF might be considered to be indicated in patients of any age with severe neutropenia due to bone marrow failure under the following circumstances:

- Proven bacterial or fungal infection unresponsive to antimicrobial therapy or probable bacterial or fungal infection unresponsive to appropriate blind antimicrobial therapy.
- Neutrophil recovery not expected for 5–7 days.
- Children and adults with a low bodyweight might be expected to show better incremental responses to granulocyte transfusions.

Granulocyte transfusions might be considered inappropriate for:

- Patients with malignant haematological disease resistant to treatment.
- Ventilated patients.

Granulocyte transfusions were previously thought to be inappropriate for patients with known HLA alloimmunisation, but a recent study found that the presence or development of white blood cell (WBC) antibodies had no demonstrable effect on any clinical aspect of granulocyte transfusion therapy [15].

Approaches to Complications Associated with Blood Transfusion in Patients with Malignant Haematological Disease

Transfusion-Transmitted Cytomegalovirus Infection

Clinical Features and Risk Factors

Cytomegalovirus (CMV) infection may cause significant morbidity and mortality in immunocompromised patients, mainly due to pneumonia. Patients who have never been exposed to CMV are at risk for primary infection transmitted by blood components prepared from blood donors who have previously had CMV infection and still carry the virus.

Patients who have been previously exposed to CMV and are CMV seropositive are at risk of reactivation of CMV during a period of immunosuppression. The extent to which CMV-seropositive patients are at risk from reinfection with different strains of CMV remains unknown, but this risk is generally considered to be very low. The patients at risk of transfusion-transmitted CMV infection are shown in Box 32.1 [16]. The use of CMV-seronegative blood components has been shown to reduce the incidence of CMV infection in groups at risk for transfusion-transmitted CMV infection to 1–3%. Incomplete prevention has been suggested to be due to:

- Occasional failure to detect low-level CMV antibodies.
- Loss of antibodies in previously infected blood donors.
- Transfusion of blood components prepared from recently infected donors.

CMV is transmitted by leucocytes, and a number of studies have found that prestorage leucocyte reduction of blood components is as effective as the use of CMV-seronegative blood components in the prevention of transfusion-transmitted CMV infection in neonates, patients undergoing remission induction therapy for acute leukaemia and after haemopoietic stem cell transplantation. These data suggest that prestorage leucocyte-reduced blood components can be accepted as a substitute for CMV-seronegative blood components for patients at risk of transfusion-transmitted CMV infection when CMV-seronegative blood components are not available, i.e. that leucocyte-reduced blood components are 'CMV safe'. In the UK, the Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) recently concluded that the range of patients

Box 32.1 Patients at risk for transfusion-transmitted cytomegalovirus (CMV) infection.

Risk well established

- CMV-seronegative recipients of allogeneic bone marrow/peripheral blood progenitor cell transplants from CMV-seronegative donors.
- CMV-seronegative pregnant women.
- Premature infants (< 1.2 kg) born to CMV-seronegative women.
- CMV-seronegative patients with human immunodeficiency virus (HIV) infection.

Risk less well established

- CMV-seronegative patients receiving autologous bone marrow/peripheral blood progenitor cell transplants.
- CMV-seronegative patients who are potential recipients of allogeneic or autologous bone marrow/peripheral blood progenitor cell transplants.
- CMV-seronegative patients receiving solid organ (kidney, heart, lung, liver) transplants from CMV-seronegative donors.

Risk not established

- CMV-seronegative recipients of allogeneic bone marrow/peripheral blood progenitor cell transplants from CMV-seropositive donors.
- CMV-seropositive recipients of bone marrow/peripheral blood progenitor cell transplants.
- CMV-seropositive recipients of solid organ transplants.

Source: Clark and Miller 2010 [16].

provided with CMV-screened blood should be reduced [17]. Specifically, it indicated that for patients with malignant haematological disease:

- CMV-seronegative red cells and platelets may be replaced with leucocyte-reduced blood components for adults and children post haemopoietic stem cell transplantation, for all patient groups including seronegative donor/seronegative recipients.
- Patients requiring transfusions who may require a transplant in the future may also safely be transfused with leucocyte-reduced products.

Transfusion-Associated Graft-Versus-Host Disease

Pathogenesis and Clinical Features

Transfusion-associated graft-versus-host disease (TA-GVHD) is a rare but serious complication of blood transfusion [18]. As discussed in Chapter 14, there is engraftment and proliferation of donor T lymphocytes and interaction with recipient cells expressing HLAs, causing cellular damage particularly to the skin, gastrointestinal tract, liver and spleen and the bone marrow. Clinical manifestations usually occur 1–2 weeks after blood transfusion, and early features include fever, maculopapular skin rash, diarrhoea and hepatitis. Haematology patients at risk are those who are undergoing transplantation, have Hodgkin lymphoma or have received therapy with certain drugs, such as purine analogues.

Prevention

The dose of donor lymphocytes sufficient to cause TA-GVHD is unknown, but may be lower than is achievable by current techniques for leucocyte reduction of blood components. Until recently the standard method to prevent TA-GVHD was γ-irradiation to destroy the proliferative capability of donor lymphocytes, although it is a radioactive source and requires regular recalibration. An alternative to γ-irradiation is X-ray irradiation, which is being increasingly used worldwide. Key considerations in the assessment of methods for the prevention of TA-GVHD are their effectiveness and the avoidance of excessive damage to red cells and platelets. The currently recommended indications for

the use of irradiated blood components for haematology patients are shown in Box 32.2 [19]. Pathogen-reduction technologies have been shown to be effective at inactivating lymphocytes. Pathogen-reduced platelet components are accepted as safe from the risk of TA-GVHD in some countries without the need for further processing; similar systems are in development for red cells, but are not yet licensed.

Ensuring That Patients Receive the Correct 'Special' Blood

An important issue for haematology departments and hospital blood transfusion laboratories is how to ensure that patients receive special blood components (e.g. irradiated) when these products are indicated and that standard blood components are not transfused, as this may have devastating consequences.

Box 32.2 Indications for irradiation of blood components in haematology patients [19].

Indications

Allogeneic haemopoietic stem cell transplantation: from the time of initiation of conditioning chemo/radiotherapy. This applies for all conditions regardless of the underlying diagnosis. Irradiated components should be continued until all of the following criteria are met:

- More than 6 months have elapsed since the transplant date.
- The lymphocyte count is $> 1.0 \times 10^9$/L.
- The patient is free of active chronic graft-versus-host disease (GVHD).
- The patient is off all immunosuppression.

If chronic GVHD is present or continued immunosuppressive treatment is required, irradiated blood components should be given indefinitely. Treatment with irradiated blood components should continue indefinitely if this is required based on transplant conditioning, underlying disease or previous treatment, e.g. previous diagnosis of Hodgkin lymphoma or previous purine analogue treatment.

Allogeneic cellular blood components transfused to bone marrow and peripheral blood stem cell donors of all ages within 7 days prior to or during the harvest should also be irradiated.

- *Autologous haemopoietic stem cell transplantation*: during and 7 days before the harvest of haematopoietic cells, and then from the initiation of conditioning therapy until 3 months post transplant (6 months if total body irradiation is used), unless conditioning, disease or previous treatment determines indefinite duration, e.g. previous diagnosis of Hodgkin lymphoma or previous purine analogue treatment.
- *Hodgkin lymphoma*: both adults and children should have irradiated red cells and platelets for life.
- *Patients undergoing peripheral blood lymphocyte collections for future chimeric antigen receptor T cell (CAR-T) therapy*: should receive irradiated cellular blood components from 7 days prior to and during the harvest, to prevent the collection of viable allogeneic T lymphocytes. Irradiated blood components should continue to be used until 3 months following CAR-T cell infusion, unless conditioning, disease or previous treatment determines indefinite duration, e.g. previous diagnosis of Hodgkin lymphoma or previous purine analogue treatment.
- *Patients receiving transfusions of human leucocyte antigen (HLA)-selected platelets, transfusion of granulocytes and cellular components and fresh plasma from first-or second-degree relatives*.
- *Patients treated with purine analogues such as fludarabine, cladribine and pentostatin and those receiving alemtuzumab (anti-CD52)*: should receive irradiated blood components indefinitely.

Each hospital needs to establish its own procedures so that patients receive the correct special blood components, where they are indicated. These procedures should include the following:

- Education of ward medical and nursing staff about the indications for special blood components and the importance of receiving the correct type of blood component.
- Requests for blood components to include the patient's diagnosis and any requirement for special blood components.
- Storing of individual patient's requirements for special blood components in the blood transfusion laboratory information management system.
- Agreement between clinical areas and transfusion laboratories about the implementation of communication processes to ensure that specific requirements and provision of irradiated cellular blood components are met for patients under shared care.
- Inclusion in the prescription for blood components any requirement for special blood components, enabling the ward staff to check that the blood component to be transfused complies with these requirements as part of the pretransfusion bedside check process.
- Providing patients with cards indicating their special blood requirements, particularly for those patients receiving shared care between two hospitals and those with a long-term requirement for irradiated blood, e.g. patients with Hodgkin lymphoma.

Human Leucocyte Antigen Alloimmunisation and Refractoriness to Platelet Transfusions

Platelet refractoriness is the repeated failure to obtain satisfactory responses to platelet transfusions and it occurs in more than 50% of patients receiving multiple transfusions.

Various methods are used to assess response to platelet transfusions. If the patient is bleeding, the clinical response is an important indication of the effectiveness of the transfusion. The response to a prophylactic platelet transfusion is assessed by measuring the increase in platelet count after the transfusion. Various formulas have been used to correct for the variation in response, dependent on the patient's size and the number of platelets transfused; these include platelet recovery and corrected count increment. However, in practice, a non-sustained increase in the patient's platelet count of less than 5×10^9/L at 20–24 hours after the transfusion can be used as a simple measure of a poor response [20].

Causes

Many causes of platelet refractoriness have been described and can be subdivided into immune mechanisms, most importantly HLA alloimmunisation and non-immune mechanisms involving platelet consumption (Box 32.3). Platelet consumption is the most frequent mechanism of platelet refractoriness, usually associated with sepsis [21]. However, immune-mediated platelet destruction remains an important cause of platelet refractoriness; HLA antibodies are

Box 32.3 Causes of platelet refractoriness.

Immune

- Platelet alloantibodies.
- Human leucocyte antigen (HLA).
- Human platelet antigen (HPA).
- ABO.
- Other antibodies.
- Platelet autoantibodies.
- Drug-dependent platelet antibodies.
- Immune complexes.

Non-immune

- Infection and its treatment, especially amphotericin B.
- Splenomegaly.
- Disseminated intravascular coagulation.
- Fever.
- Bleeding.

the most common immune cause and the other immune causes are rare.

The precise mechanism of HLA alloimmunisation remains uncertain, but primary HLA alloimmunisation appears to be initiated by intact cells expressing both HLA class I and class II antigens, such as lymphocytes and antigen-presenting cells. Platelets only express HLA class I antigens and hence leucocyte-reduced blood components cause primary HLA alloimmunisation in fewer than 3% of recipients. Use of prestorage leucocyte-reduced blood components resulted in a significant reduction in the incidence of HLA alloimmunisation [22]. However, secondary HLA alloimmunisation does not require the presence of HLA class II antigens, and may occur in patients who have been pregnant or previously transfused with non-leucocyte-reduced blood components.

Investigation and Management

If platelet refractoriness occurs, the algorithm in Figure 32.1 can be used for investigation and management.

1) A clinical assessment should be made for clinical factors likely to be associated with non-immune platelet consumption.
2) If non-immune platelet consumption appears likely, an attempt should be made to correct the clinical factors responsible, where possible, and platelet transfusions from random donors should be continued. If a poor response to random donor platelet transfusions persists, the patient should be tested for HLA antibodies.
3) If non-immune platelet consumption appears to be unlikely, an immune mechanism should be suspected and the patient's serum should be tested for HLA antibodies. If HLA antibodies are present, the specificity of the antibodies should be determined, as this may help in the election of HLA-compatible donors. However, HLA antibodies stimulated by repeated transfusions are often 'multispecific' and it is not possible to determine their specificity.
4) Platelet transfusions from HLA-matched donors (matched for the HLA-A, -B

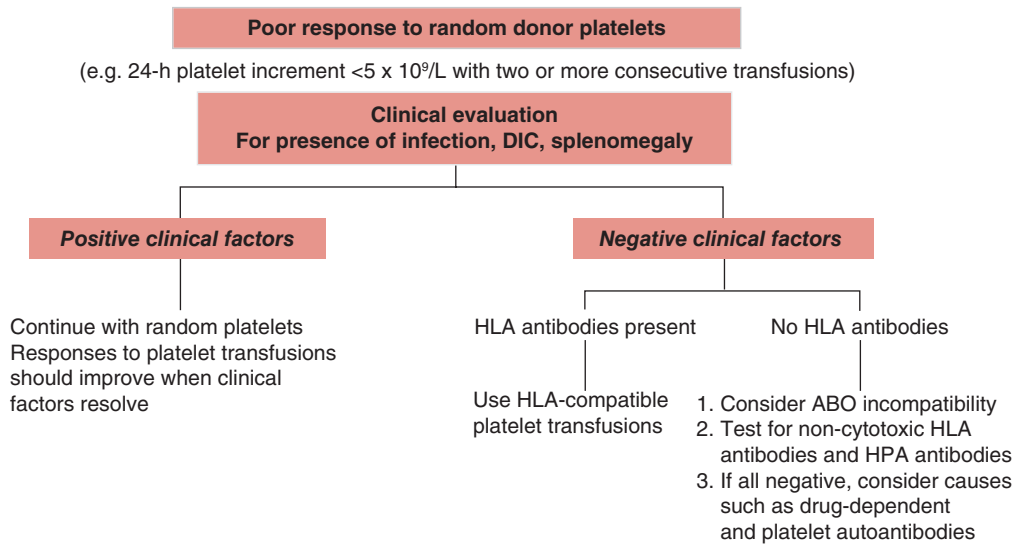

Figure 32.1 Algorithm for the investigation and management of patients with platelet refractoriness. DIC, disseminated intravascular coagulation; HLA, human leucocyte antigen; HPA, human platelet antigen. *Source:* Adapted from Phekoo et al. 1997 [23].

antigens of the patient) should be used for patients with apparent immune refractoriness and the response to further transfusions should be observed carefully. Figure 32.2 shows improved responses to HLA-matched platelet transfusions in a patient with platelet refractoriness due to HLA alloimmunisation. If responses to HLA-matched transfusions are not improved, the reason should be sought, and platelet crossmatching of the patient's serum against the lymphocytes and platelets of HLA-matched donors may be helpful in determining the cause and in the selection of compatible donors for future transfusions. These matching strategies are based on counting the number of HLA-A and -B mismatches between the patient and donor; this requires a large HLA-typed donor panel and at times no suitable matches can be found. An alternative approach is HLA epitope matching; this only considers the epitopes on the HLA antigen, whereas standard HLA matching considers the whole HLA antigen.

5) If there are no factors for non-immune platelet consumption and HLA antibodies are not detected, consideration should be given to less frequent causes of immune platelet refractoriness:

- High-titre ABO antibodies in the recipient. This is an unusual cause of platelet refractoriness and can be excluded by switching to ABO-compatible platelet transfusions if ABO-incompatible transfusions have been used for previous transfusions.
- Human platelet antigen (HPA) antibodies, which usually occur in combination with HLA antibodies, but sometimes occur in isolation.
- Drug-dependent platelet antibodies, which may be underestimated as a cause for platelet refractoriness.

Alloimmunisation to Red Cell Antigens

Incidence

Alloimmunisation to red cell antigens is another important consequence of repeated transfusions in haematology patients. The incidence of red cell alloimmunisation in patients with malignant haematological disease is in the range of 10–15% and is similar to other groups of multitransfused patients, such as those with renal failure.

Pheno/genotyping and antigen matching to prevent red cell alloimmunisation are not required for patients with malignant haematological disease requiring repeated

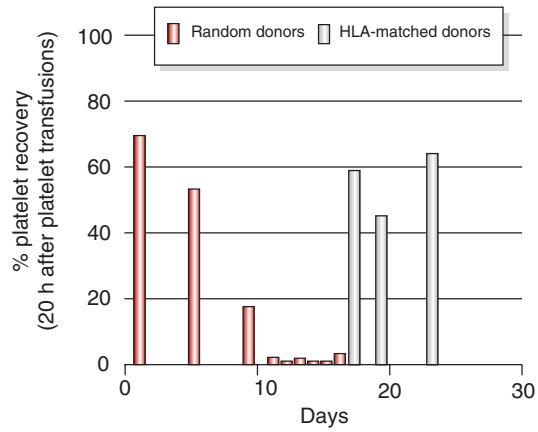

Figure 32.2 Responses to platelet transfusions in a female patient with acute myeloblastic leukaemia undergoing remission induction therapy. There were poor responses to the initial platelet transfusions and the patient was found to have human leucocyte antigen (HLA) antibodies. There were improved responses to platelet transfusions from HLA-matched donors.

transfusions, except for those at increased risk such as transfusion-dependent patients on chronic transfusion programmes and those treated with new monoclonal antibody therapies, such as anti-CD38 and anti-CD47, known to interfere with compatibility testing.

Timing of Sample Collection for Compatibility Testing

In patients with haematological disorders receiving repeated transfusions, an important issue is the timing of blood sample collection in relation to the previous transfusion [24].

- Where the patient is receiving very frequent transfusions, for example daily, it is only necessary to request a new sample every three days.
- In the UK, if the previous transfusion was 3–14 days earlier, the sample should ideally be taken within 24 hours of the start of the transfusion, although some laboratories stretch this to 48 hours for patients who have been repeatedly transfused without developing antibodies. Other countries, such as Canada, only require a sample within 3 days of the start of the transfusion.
- Where the previous transfusion was 14–28 days earlier, the sample should be taken within 3 days of the start of the transfusion.
- Where the previous transfusion was more than 28 days ago, the sample should be taken within one week of the planned transfusion.

Sample collection timeframe requirements do vary slightly from country to country.

ABO-Incompatible Bone Marrow/Peripheral Blood Progenitor Cell Transplants

ABO-incompatible bone marrow/peripheral blood progenitor cell transplants present particular problems (Box 32.4). The transplant may provide a new A and/or B antigen from the donor (major mismatch) or a new A and/or B antibody (minor mismatch). For *pretransplant* transfusions, blood component support should be with the patient's own ABO type. For *posttransplant* transfusions, selection of the appropriate group is more complicated (Figure 32.3) [25]. These recommendations should be followed post transplant until the patient has engrafted,

Box 32.4 Problems associated with ABO-incompatible bone marrow/peripheral blood progenitor transplants.

Major ABO incompatibility (e.g. recipient O, donor A)

- *Failure of engraftment*: risk not increased in ABO-incompatible transplants.
- *Acute haemolysis at the time of reinfusion*: avoided by processing donor bone marrow/peripheral blood progenitor cells.
- *Haemolysis of donor-type red cells*: avoid by using red cells of recipient type in the early posttransplant period.
- *Delayed erythropoiesis*: may be due to persistence of anti-A in the recipient; minimise transfusion of anti-A by using platelets and plasma from group A donors.
- *Delayed haemolysis due to persistence of recipient anti-A*: only switch to donor red cells when recipient anti-A undetectable and direct antiglobulin test undetectable.

Minor ABO incompatibility (e.g. recipient A, donor O)

- *Acute haemolysis at the time of reinfusion*: avoid by removing donor plasma if the donor anti-A titre is high.
- *Delayed haemolysis of recipient cells due to anti-A produced by donor lymphocytes (passenger lymphocyte syndrome)*: maximum haemolysis usually occurs between days 9 and 16 post transplant. Rare in T-cell-depleted grafts or when CD34+ cells selected in stem cell processing.

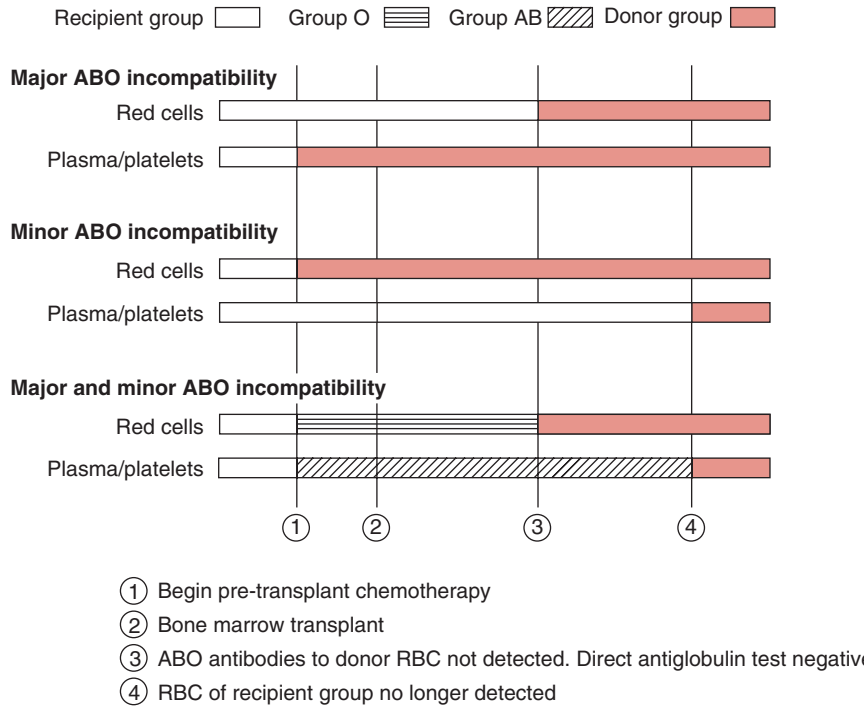

Figure 32.3 Recommendations for ABO type of blood components in ABO-incompatible bone marrow/peripheral blood progenitor cell transplants. RBC, red blood cell. *Source:* Warkentin 1983 [25], reproduced with permission of Elsevier.

ABO antibodies to the donor ABO group are undetectable and the direct antiglobulin test is negative.

- *Major ABO incompatibility*: the patient's own ABO group should be given. Plasma and platelets should be of the donor-type blood group. The European Group for Blood and Marrow Transplantation (EBMT) also advises that group O red cells can be used.
- *Minor ABO incompatibility:* red cells of the donor ABO group should be given. Plasma and platelets should be of a recipient-type blood group.
- *Major and minor (bidirectional) ABO incompatibility*: give group O red cells, group AB plasma and platelets of the recipient-type blood group.

Studies disagree on whether ABO incompatibility can also affect overall survival, disease-free survival or GVHD. A meta-analysis [26] found no difference in overall survival between recipients of ABO-matched or ABO-mismatched grafts when the donor was related. However, in unrelated donor transplants there was a marginally reduced overall survival in patients who received bidirectional or minor mismatched transplants.

Rhesus D-incompatible transplants can also cause difficulties. It is recommended that RhD-negative blood components should be used for RhD-positive recipients with RhD-negative donors. However, no cases of immunisation have been reported when RhD-negative recipients have received RhD-positive transplants, and RhD-positive blood components may be used.

Iron Overload

A major adverse consequence of repeated red cell transfusions over a long period in is iron overload. This important complication is described in detail in Chapter 30.

KEY POINTS

1) Specialist transfusion support and advice are required for many patients with malignant haematological disorders.
2) The need for transfusion, as in other groups of patients, is determined by assessment of individual patient symptoms and blood counts and guided by national and local recommendations for the use of blood.
3) Special blood components are frequently needed to avoid complications such as transfusion-associated graft-versus-host disease (TA-GVHD).
4) Responses to platelet transfusions should be carefully monitored to identify patients with poor responses, which require clinical and laboratory investigation to determine the most likely cause and the best approach to management.
5) Further work is needed to define the optimal thresholds for red cell and platelet transfusion in patients with haematological malignancies and the role of granulocyte transfusions.

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33 Transfusion in Benign Haematological Disease

Eric Salazar[1] *and Christopher A. Tormey*[2]

[1] *Department of Pathology & Genomic Medicine, Houston Methodist Hospital, Houston, TX, USA*
[2] *Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA*

Benign haematological disorders frequently create problems for the blood bank, be they serological/testing challenges or obstacles to transfusion therapy. The aim of this chapter is to provide a practical overview of common, non-malignant haematological disorders relevant to transfusion practice. For each entity/disorder, we will review pathophysiology, approaches to transfusion therapy and possible alternative treatments. Our focus is benign red blood cell (RBC) and platelet (PLT) disorders, with a particular emphasis on acquired immunological and functional diseases. Congenital disorders of haemoglobin are covered elsewhere, as are congenital/acquired (non-PLT-related) bleeding problems.

Benign Red Blood Cell Disorders

Acquired Disorders of Haemolysis

Autoimmune Haemolytic Anaemias

Autoimmune haemolytic anaemias (AIHAs) result from autoantibodies directed against erythrocytes. AIHAs are classified based on optimal autoantibody binding temperature in serological assays. Warm autoantibodies react stronger at 37 °C and are immunoglobulin (Ig)G, while cold autoantibodies react stronger at 4 °C and tend to be IgM. Autoantibodies detected in RBC serological assays can cause a spectrum of clinical sequalae, ranging from no clinical significance to accelerated RBC clearance by macrophages in the spleen and liver. Others fix complement and cause rapid intravascular haemolysis. This distinction can be important from a treatment perspective [1]. Table 33.1 summarises AIHA serological findings and disease associations.

Warm Autoimmune Haemolytic Anaemia

Warm autoantibodies can arise in the context of lymphoproliferative disorders (e.g. chronic lymphocytic leukaemia) or autoimmunity, or they can be idiopathic. Serological assays typically show panreactivity in both direct and indirect antiglobulin tests (DAT and IAT, respectively), with a positive autologous control, reflective of broad specificity. Occasionally, warm autoantibodies can show specificity for RBC antigens, most commonly Rh system antigens.

Warm autoimmune haemolytic anaemia (WAHA) can be a medical emergency requiring emergency ABO-compatible transfusion. RBC administration should not be delayed in the context of hypoxia, altered mental status or haemodynamic instability [1]. As time allows, the blood bank should test for clinically significant underlying alloantibodies to guide RBC unit selection. Adsorption of the serum to remove the panreactivity is usually

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Table 33.1 Typical serological properties and disease associations in autoimmune haemolytic anemia (AIHA).

AIHA type	DAT results		Autoantibody class	Autoantibody specificity	Common associations in secondary forms
	IgG	C3			
Warm*	+	–	IgG	Variable; may be Rh-like (e, C)	Mixed connective tissue diseases; viral infection; B-NHL
CAD	–	+	IgM	I; i†	*Mycoplasma pneumoniae* infection†; EBV infection†; B-NHL
PCH	–	+	IgG	P	Viral infection; post vaccination

* Note that up to 25–30% of warm AIHA can also show C3+ DAT studies.
† *Mycoplasma pneumoniae* infection is most often associated with auto-anti-I specificity, while EBV infection is most often associated with auto-i specificity.
B-NHL, mature B-cell non-Hodgkin lymphoma; CAD, cold agglutinin disease; DAT, direct antiglobulin test; EBV, Epstein–Barr virus; Ig, immunoglobulin; PCH, paroxysmal cold haemoglobinuria.

required. If the patient has not been recently transfused, autoadsorption with autologous RBCs should be carried out as appropriate; collection of numerous tubes of autologous blood for testing should be done judiciously in the context of severe anaemia. Autologous RBCs are first treated with ZZAP, a reagent that removes already bound autoantibodies, increasing the adsorption potential of the autologous cells [2]. If the patient has been recently transfused, alloadsorption with phenotypically matched RBCs can be performed with serum/plasma, then tested via IAT to identify any underlying alloantibodies.

Since patients with WAHA may require frequent transfusion, provision of phenotypically matched RBCs is advised. In addition, warm autoantibodies often cause a positive crossmatch, necessitating transfusion of crossmatch-incompatible RBCs [1]. A common approach is to issue RBC units that are Rh and Kell matched, negative for any antigens associated with corresponding alloantibodies, and that are 'least incompatible' (have the weakest crossmatch reaction) [3]. RBC units that are crossmatch incompatible can raise safety concerns among the clinical staff and close communication with the blood bank is important.

Cold Agglutinin Disease

Clinically insignificant cold autoantibodies, reacting at 4 °C, are frequently detected in healthy patients. Some cold autoantibodies may have a wider thermal amplitude, reacting at higher temperatures. Cold agglutinin disease (CAD) results when cold autoantibodies with a wide thermal amplitude and high titre bind to RBCs in cooler parts of the body (extremities, etc.) and then fix complement when the RBCs circulate to the warmer body core. Intravascular or extravascular haemolysis may occur. Like warm autoantibodies, pathological cold autoantibodies may arise in the context of malignancy, autoimmune disease or infection, or may be idiopathic.

Cold agglutinins often have identifiable specificity with auto-anti-I (the most common cause of cold agglutinin disease), associated with *Mycoplasma pneumoniae* infection, and auto-anti-i, associated with mononucleosis. Auto-anti-P, the cause of paroxysmal cold haemoglobinuria, can arise following viral infections in children [4].

The serological findings in CAD usually include a positive DAT with anti-C3. Depending on the antibody thermal amplitude, a cold agglutinin may cause panreactivity in antibody workups, like warm autoantibodies, and if this is the case it may be necessary to unmask underlying alloantibodies. Cold agglutinins can interfere with ABO/Rh typing and crossmatching. Various techniques attenuate cold agglutinin reactivity. These include prewarming the sample, adsorption of the test

serum with rabbit erythrocyte stroma (RESt), which binds IgM cold antibodies, or treatment of serum/plasma with reducing agents such as dithiothreitol, which destroy pentameric IgM [4]. Investigation of the clinical significance of a cold agglutinin may require determination of the cold antibody titre and thermal amplitude [1].

Clinically significant cold agglutinins can pose major challenges. If possible, transfusion is avoided. The patient should be kept warm, and any infusing fluids, including blood transfusions, should be passed through a fluid/blood warming device. Strong cold agglutinins can cause RBC clumping in laboratory samples, interfering with laboratory testing, especially automated haematology analysers. Therefore, samples may need to be kept warm or prewarmed prior to testing.

Paroxysmal Cold Haemoglobinuria

Paroxysmal cold haemoglobinuria (PCH) occurs most often in children. Patients may complain of chills, fever and haemoglobinuria following exposure to cold temperatures. Auto-anti-P is the most common cause of PCH. The auto-anti-P in PCH is a biphasic IgG that binds to the P antigen at colder temperatures and fixes initial components of complement cascade [5]. At warmer temperatures the IgG antibody disassociates, but the remaining complement proteins are activated, resulting in acute intravascular lysis. The DAT typically is IgG negative, but C3 positive. The biphasic haemolytic antibody is diagnosed with the Donath–Landsteiner test, in which *in vitro* haemolysis is observed after incubating the patient's serum with fresh complement and reagent group O RBCs on melting ice and transferring the sample to 37 °C. Relative to control samples kept on melting ice or at 37 °C, haemolysis is observed only in the sample kept on ice and then transferred to 37 °C [2].

Patients with PCH should be kept warm, and intravenous fluids and transfusions should be administered with a warming device [6]. Since the antibody does not bind at 37 °C, the IAT and crossmatch are typically negative. Even though the antibody has specificity for anti-P, transfusion with P-negative RBCs, which are difficult to procure, is not necessary.

Paroxysmal Nocturnal Haemoglobinuria

Paroxysmal nocturnal haemoglobinuria (PNH) is caused by an acquired clone of haematopoietic stem cells with a phosphatidylinositol glycan A (PIGA) enzyme mutation. PIGA is essential in the formation of glycosyl phosphatidylinositol (GPI) cell membrane anchors that anchor many important cell membrane-bound proteins. All progeny of the defective clone, including erythrocytes, granulocytes and platelets, will have the defect. GPI-anchored proteins include complement inhibitory proteins, rendering defective RBCs susceptible to complement-mediated haemolysis. Platelets with absent GPI-anchored proteins also cause thrombotic complications. Modern diagnosis of PNH relies on flow cytometry of RBCs stained for the GPI-anchored proteins CD55, CD58 and CD59, or with fluorescein-labelled proaerolysin variant (FLAER), which binds directly to GPI anchors [7].

Since most of the manifestations of PNH result from complement dysregulation, anti-complement therapy with eculizumab is now standard of care. Eculizumab results in improved haemoglobin level, fewer RBC transfusions and improved markers of haemolysis [8]. When transfusion is required, special modifications, like washed RBCs, provide no additional benefit and are not required.

Congenital Disorders of Haemolysis

Enzyme- and Membraneopathies

The most common enzyme abnormality causing haemolysis is glucose-6-phosphate dehydrogenase (G6PD) deficiency, which limits protection of RBCs from oxidative stress. Stressors, such as infections, certain medications and certain foods (e.g. fava beans), can increase oxygen free radicals, oxidising and denaturing haemoglobin into precipitated Heinz bodies. Heinz bodies adhere to the cell membrane and render RBCs more rigid and fragile [9].

G6PD deficiency is usually managed with avoidance of oxidative stressors. Transfusion

is rarely indicated in the context of symptomatic anaemia. If the patient is likely to receive frequent transfusions, units phenotypically matched for clinically significant antigens may be warranted. Transfusion medicine practitioners should also be aware that RBC donors are not typically screened for G6PD deficiency [9]. Generally the risk to transfusion recipients is only theoretical, but may be clinically relevant in the context of exchange transfusion of newborn infants [10].

Haemolytic RBC membrane disorders arise from mutations affecting the proteins that control RBC shape and deformability. These proteins include spectrin, ankyrin, protein 4.2, protein 4.1, band 3 protein and glycophorin C [11]. Mutations affecting these proteins can cause morphological findings on the peripheral smear forming the basis for disease categorisation, including hereditary spherocytosis, eliptocytosis and stomatocytosis. Haemolysis, usually extravascular, can result from the weakened mechanical stability of cell membrane. The degree of haemolysis can vary significantly, and splenectomy may be helpful in some cases [11]. As with any patient likely to receive recurrent transfusions, RBCs phenotypically matched for common clinically significant antigens would likely be indicated to mitigate the risk of alloimmunisation.

Congenital and Acquired Microangiopathic Disorders

There are several causes of fragmentation haemolysis, in which extracellular factors create RBC fragments (schistocytes). Fragmentation haemolysis and anaemia produced by microthrombi present in the microvasculature are referred to as microangiopathic haemolytic anaemia (MAHA). When pathological microthrombi cause consumptive thrombocytopenia, MAHA and end-organ damage, this is considered thrombotic microangiopathy (TMA). The classic TMAs include immune-mediated thrombotic thrombocytopenic purpura (TTP), congenital TTP (Upshaw–Schulman syndrome), infection-related haemolytic-uremic syndrome (HUS), atypical HUS and disseminated intravascular coagulation (DIC). Accurate diagnosis of these entities is critical, since these are potentially immediately life-threatening entities with different treatments (Table 33.2).

Immune-Mediated Thrombotic Thrombocytopenic Purpura and Upshaw–Shulman Syndrome

TTP is cause by a severe deficiency of ADAMTS-13, an enzyme that cleaves von Willebrand factor (vWF) multimers. Ultra-large vWF multimers persist and these can cause PLT consumption and microthrombus formation, with PLT counts decreasing to very low levels. Severe ADAMTS-13 deficiency can result from acquired autoantibody inhibitors or can be due to inherited mutations (Upshaw–Schulman syndrome). Definitive diagnosis of TTP requires assessment of the ADAMTS-13 level and testing to detect inhibitors.

Acquired TTP requires prompt recognition and initiation of therapy, or it is almost always fatal. The PLASMIC score may help in distinguishing TTP from other TMAs [12]. A presumptive or suspected diagnosis is sufficient to initiate therapy, while definitive testing is conducted. Treatment includes therapeutic plasma exchange (TPE) with plasma replacement, immunosuppression and, in selected patients, caplacizumab [13]. In acquired TTP, TPE decreases overall mortality to < 10–20% and is typically performed daily with plasma as the replacement fluid until PLT counts and markers of haemolysis normalise [14]. TPE with plasma replacement is thought to remove anti-ADAMTS13 autoantibodies while replacing ADAMTS13 activity. Some patients develop allergic reactions due to multiple plasma transfusions, which may be mitigated with solvent-detergent plasma. Patients with congenital TTP usually require chronic plasma transfusion. PLT transfusion is generally avoided in TTP, unless absolutely required, due to risk of thrombosis [15].

Infectious Haemolytic-Uremic Syndrome and Atypical Haemolytic-Uremic Syndrome

Most cases of infectious haemolytic-uremic syndrome (iHUS), also referred to as typical HUS or diarrhoea-associated HUS, arise in the context of infection with Shiga toxin-producing

Table 33.2 Summary of similarities and differences in clinical and laboratory findings, as well as treatment modalities, among common forms of thrombotic microangiopathy (TMA).

Disorder	Primary mechanism of TMA	Key diagnostic features	Therapeutic approach
TTP	ADAMTS-13 deficiency (congenital or acquired)	Very low platelet count ADAMTS-13 level very low or absent	Plasma exchange + immunosuppression (acquired) Plasma infusion (congenital)
iHUS	Bacterial infection	Microbiology tests positive Prominent renal failure	Supportive care +/– haemodialysis
aHUS	Complement dysregulation (congenital or acquired)	Low platelet count Prominent renal failure ADAMTS-13 level moderate to normal	Complement inhibition (congenital) Plasma exchange (acquired)
DIC	Underlying inflammatory disease or malignancy	Abnormal coagulation tests (PT and PTT prolonged, fibrinogen decreased, elevated D-dimer)	Transfusion + treatment of underlying disease

aHUS, atypical haemolytic uremic syndrome; DIC, disseminated intravascular coagulation; iHUS, infection-associated haemolytic uremic syndrome; PT, prothrombin time; PTT, partial thromboplastin time; TTP, thrombotic thrombocytopenic purpura.

bacteria and/or *Streptococcus pneumoniae*. It is thought that Shiga toxins and locally released cytokines cause the release of ultra-large multimers of vWF, leading to microthrombi formation, especially in the renal vasculature. Pneumococcus produces an enzyme that exposes T antigen on RBCs, platelets and glomeruli, and naturally occurring anti-T-antigen antibodies lead to pathogenic T activation and HUS [16]. Treatment of iHUS includes treatment of the underlying infection, supportive transfusions and haemodialysis, as needed.

Also a complement-mediated disorder, atypical haemolytic-uremic syndrome (aHUS) arises in patients with mutations in complement regulatory genes including factor H, factor I, membrane co-factor protein and factor B. Some aHUS patients have autoantibodies against factor H. Defects in complement regulation allow for chronic, uncontrolled activation of the complement system and systemic TMA, often with prominent renal failure. Even in patients with complement regulatory mutations, aHUS can present in adulthood. Commonly, in patients presenting with a rapidly progressing TMA the distinction between TTP and aHUS cannot be made. TPE may be initiated until ADAMTS13 testing is completed. It is important to draw the samples for ADAMTS13 testing prior to TPE initiation. If TTP is excluded and aHUS is suspected, treatment with anti-complement therapy, eculizumab, is indicated. The benefit of TPE in most cases of aHUS is unclear, but TPE is indicated in the context of factor H autoantibody [14]. Eculizumab in aHUS is associated with improvement in renal function and fewer thrombotic events [17].

Disseminated Intravascular Coagulation

DIC is a consumptive coagulopathy with potential to cause thrombosis and/or haemorrhage. Triggers include infection, inflammation and malignancy, and the presentation varies from chronic to life-threatening. In contrast to other TMAs, DIC presents with abnormal coagulation studies in addition to thrombocytopenia. D-dimer is usually elevated. Management of DIC includes identification and treatment of the underlying cause, and individualised transfusion support in bleeding patients. In patients with life-threatening bleeding, PLT transfusion to maintain a count > 50 000/μL is appropriate [18]. Similarly, patients with life-threatening bleeding and significantly prolonged prothrombin time and/or significantly decreased

fibrinogen level should receive plasma transfusion and/or cryoprecipitate transfusion. Transfusion thresholds should be individualised for the clinical scenario. Unless there is a primary hyperfibrinolytic state, bleeding patients should not be treated with antifibrinolytic agents. Prothrombin complex concentrate is generally contraindicated in DIC patients.

Benign Platelet Disorders

A variety of PLT disorders are encountered in transfusion/blood bank practice. In this section, we will review common functional as well as immunological disorders of PLTs.

Congenital Disorders of Platelet Function

Qualitative Platelet Disorders

Although it is rare, some individuals possess hereditary PLT function defects wherein loss/malfunction of surface receptors, abnormalities in signalling pathways or absence of granules leads to bleeding, ranging from minor to life-threatening [19]. The most common of these entities are described in Table 33.3 – they all can be diagnosed and/or monitored via traditional tests (e.g. aggregometry) as well as more novel platforms [20].

For functional defects, PLT transfusion is a reasonable option for treating bleeding, or for procedural prophylaxis. Since most hereditary disorders do not significantly impact PLT counts, goals of transfusion therapy are based on objective measurements of function or, more commonly, are empirically derived. At the authors' facilities, and in the absence of objective functional testing, we apply an approach not dissimilar from quantitative PLT problems. For mild–moderate bleeds (non-neuraxial) or moderate- to high-risk invasive procedures we recommend starting with a single dose of PLTs, while for neuraxial bleeds or procedures we recommend starting with two PLT doses. Ongoing PLT infusions may be required for complex problems or chronic issues.

For individuals with severe congenital PLT defects, particularly those requiring chronic PLT transfusion, a major concern is PLT alloimmunisation [21]. Should a patient develop immune-mediated PLT refractoriness, crossmatch-compatible or antigen-matched units may be required. If these are not readily available, or if patients bleed despite PLT transfusion, recombinant factor VIIa may be useful, particularly in Glanzmann's thrombasthenia [22].

Acquired Disorders of Platelet Function

Drug-Induced Thrombocytopathy

A variety of drugs impact PLT function. While PLT inhibition is the goal of such therapy, individuals on anti-PLT medications can be at risk for severe bleeding, occurring spontaneously or in the setting of trauma or surgery [23]. Table 33.4 details commonly used drugs with their associated mechanisms and half-lives; this information is of importance in determining an appropriate reversal strategy.

When confronted with a bleeding or surgical patient on anti-PLT drug(s), PLT count is typically irrelevant – such medications impact function, not quantity. As with congenital disorders, aggregometry tests can assess PLT inhibition, but are unlikely to be available urgently. Alternatively, more rapid assays such as adhesion-aggregation platforms (e.g. PFA-100), whole blood 'point-of-care' aggregometers (e.g. VerifyNow) and even modified viscoelastic tests (e.g. PLT mapping) may provide specific insight into the degree of PLT inhibition and whether reversal of an anti-PLT drug is needed [20].

Once anti-PLT reversal is deemed appropriate, PLT transfusion is an excellent option. Empirical approaches are most practical, although use of functional tests is becoming an increasingly popular 'targeted' option [20]. Similar to the strategy for congenital defects discussed earlier, the authors recommend starting with a single dose of PLTs for bleeds or non-neuraxial procedures.

While PLT transfusion is an 'antidote' to anti-PLT therapies, administration of PLTs is not without risks. For instance, the PATCH study – a randomised trial in adults with intracerebral haemorrhage (ICH) and on

Table 33.3 Congenital disorders of platelet (PLT) function and their associated clinical and laboratory properties.

Disorder	Primary defect	Usual severity of bleeding	Most common aggregometry findings
Glanzmann's thrombasthenia	Loss or defect in GPIIb/IIIa receptor	Moderate to severe	Absent primary/secondary response to all agonists (except ristocetin)
Bernard–Soulier syndrome	Loss or defect in GPIb/IX/V receptor complex	Moderate to severe	Absent response to ristocetin; normal responses to all other agonists
PLT-type von Willebrand disease	Defect in GPIb/IX/V complex	Mild to moderate	Increased activity with low-dose ristocetin; normal responses to all other agonists
Storage pool disease (primary and secondary)	Reduction or absence of dense granules	Mild to moderate	Absent secondary wave response to all agonists (except ristocetin)
Aspirin-like defect	Loss of function in of thromboxane synthesis pathways	Mild to moderate	Blunted to absent response to arachidonic acid, with blunted responses among other agonists (except ristocetin)
Grey platelet syndrome	Reduction or absence of alpha granules	Mild to moderate	Absent secondary-wave response to all agonists (except ristocetin)
ADP receptor defect	Loss of function or signalling in $P2Y_{12}$	Mild to moderate	Blunted to absent response to ADP; little change among other agonists
Collagen receptor defect	Loss of function or signalling in GPVI	Mild	Blunted to absent response to collagen; little change among other agonists

ADP, adenosine diphosphate; GP, glycoprotein; PLT, platelet.

anti-PLT medications – found that recipients of PLTs had *worse* outcomes than the standard therapy group, with a higher rate of death or dependence [24]. As such, PLT transfusion in ICH associated with anti-PLT therapy should be done judiciously. Desmopressin/DDAVP is a reasonable alternative option and likely enhances PLT haemostasis via release of vWF [25].

Uremic Platelet Dysfunction

Individuals with uraemia secondary to renal insufficiency can acquire PLT function defects and be at risk for bleeding. From a mechanistic standpoint, uremic PLT dysfunction reflects a spectrum of problems: reduced responsiveness to agonist-induced activation, suppressed synthesis of procoagulants and perturbation of PLT–endothelial wall interactions [26].

Uraemia-associated defects can only be definitively overcome with improvement of kidney function and/or renal replacement therapy [27]. Alternative strategies are thus warranted. Notably, PLT transfusion for uremic bleeding is *ineffective*, as allogeneic PLTs quickly acquire uraemic defects. Haemostasis therapy is instead aimed at overcoming acquired deficits by augmenting available vWF. This includes administration of either desmopressin/DDAVP (1–2 doses) and/or cryoprecipitate (once DDAVP efficacy has been exhausted, or for patients who cannot tolerate DDAVP). Medical oestrogens may also be effective [27].

Acquired Immune-Mediated Platelet Disorders

Immune Thrombocytopenic Purpura and Drug-Induced Thrombocytopenia

When exposed to a pathogen or drug/metabolite, patients can mount antibody responses against these foreign molecules.

Table 33.4 Common anti-platelet (PLT) medications and their associated properties.

Irreversible* anti-PLT agents		
Drug name	*Primary mechanism of action*	*Plasma elimination half-life (hrs)*[†]
Aspirin	COX pathway inhibition	3–6
Clopidogrel (Plavix)	$P2Y_{12}$ receptor inhibitor	5–6
Prasugrel (Effient®)	$P2Y_{12}$ receptor inhibitor	6–7
Vorapaxar (Zontivity)	Thrombin receptor inhibitor	~192–200[‡]
Reversible* anti-PLT agents		
Generic drug name	*Primary mechanism of action*	*Plasma half-life (hrs)*
Ibuprofen (Advil®, Motrin®)	COX pathway inhibition	3–4
Naproxen (Aleve®, Naprosyn)	COX pathway inhibition	3–4
Ticagrelor (Brilinta®)	$P2Y_{12}$ receptor inhibitor	7–9
Diclofenac (Voltaren)	COX pathway inhibition	2–4

* Irreversible agents are those that exert an essentially permanent effect on PLT function for the entirety of their circulating time (about 9–10 days), while reversible agents are those whose effects wane over 4–5 drug half-lives.
† Elimination half-lives provided are approximates and dose dependent, with higher doses typically being associated with longer half-lives. Note that for drugs in the irreversible category, even though half-life is limited to a few hours, their effects on circulating PLTs are permanent and do not wane as the drug clears from circulation.
‡ Although considered by some sources as a reversible anti-PLT medication, we have classified vorapaxar as an essentially irreversible PLT inhibitor given its extremely long half-life.
COX, cyclooxygenase.

In subsets of patients, antibodies generated recognise self-epitopes on PLTs, resulting in immune-mediated PLT clearance. This destruction, which typically manifests days to weeks after pathogen/drug exposure, can be severe, with PLT counts dropping to < 10 000/μL [28].

Approaches for overcoming immune-mediated PLT destruction include watchful waiting (often done in children), immunosuppressive medications and ceasing any offending medications [28]. In general, PLT transfusion is *not* indicated in patients responsive to medical management without evidence of significant/life-threatening bleeding.

For patients with immune-mediated PLT destruction who develop major haemorrhage, or require invasive procedures, PLT transfusion is warranted, although allogeneic PLTs are likely to be cleared by circulating autoantibodies. One approach to improve count increments is to provide steady infusion of PLTs in conjunction with intravenous immunoglobulin [29]. If bleeding persists, then consideration can be given to administration of a thrombopoietin mimetic. Use of anti-fibrinolytic medications in this setting may also be of value [28].

Conclusion

Benign haematological disorders present unique challenges for blood banks, from the perspectives of testing complexities as well as transfusion management. With the items discussed in this chapter, readers should possess a better sense of disease pathophysiology, as well as be armed with practical strategies for test interpretation and component therapy across the spectrum of benign RBC and PLT disorders.

KEY POINTS

1) Benign haematological disorders of red blood cells (RBCs) and platelets (PLTs) present many complexities from a testing as well as transfusion management standpoint.
2) Patients with autoimmune haemolytic anaemia frequently possess autoantibodies that interfere with pretransfusion compatibility testing and may require administration of 'least crossmatch-incompatible' RBC units, particularly those with warm-reactive autoantibodies.
3) Although the practice is controversial, patients with active cold autoimmune haemolytic anaemia (e.g. cold agglutinin syndrome and paroxysmal cold haemoglobinuria) may benefit from RBC units administered via an approved blood warmer.
4) Individuals with congenital and acquired microangiopathies often benefit from RBC, plasma and/or cryoprecipitate transfusion; however, routine PLT administration should be avoided in some of these entities (e.g. thrombotic thrombocytopenia purpura) due to increased risk for thrombosis.
5) Many congenital (rare) and acquired (common) PLT function defects can be treated by PLT transfusion, although some data suggest poorer outcomes with this practice in select settings (e.g. intracranial haemorrhage associated with anti-platelet therapy).
6) Quantitative testing (e.g. PLT count) is not useful in assessing transfusion needs for functional defects, and decisions to transfuse PLTs should be based on the clinical picture and/or results of qualitative/functional PLT analysis.
7) PLT transfusion is not efficacious in overcoming acquired functional defects associated with uraemia; therapies to address renal disease and/or increase von Willebrand factor are preferred.
8) Routine PLT transfusion is not warranted for thrombocytopenia associated with immune-mediated platelet destruction, but should be attempted when patients experience severe/life-threatening bleeds, or require high-risk invasive procedures.

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Further Reading

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34 Transfusion in Patients with Haemoglobinopathies

Yvette C. Tanhehco[1] *and Patricia A. Shi*[2]

[1] *Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY, USA*
[2] *Clinical Services, New York Blood Center; Division of Hematology, Department of Medicine, Albert Einstein College of Medicine; Division of Hematology-Oncology, Department of Medicine, Northwell Health, New Hyde Park, New York, NY, USA*

Haemoglobinopathies are congenital blood disorders affecting red blood cells (RBCs), caused by mutations in the globin genes of haemoglobin, α-globin or β-globin. About 5% of the world's population carry a significant haemoglobin variant, and about 1% of couples are at risk for having children with a haemoglobin disorder [1]. Normal adult haemoglobin consists of two α-globin and two β-globin chains ($\alpha_2\beta_2$). Quantitative defects in globin chain synthesis cause thalassaemia, while qualitative defects result in haemoglobin variants. Mutations in the α-globin gene cause α-thalassaemia syndromes, while mutations in the β-globin gene cause sickle cell disease (SCD) or β-thalassaemia syndromes. The most prevalent haemoglobin variant is haemoglobin S (HbS, β^6 glutamic acid-valine), which causes SCD in homozygotes or compound heterozygotes who also have other β-globin mutations. Blood transfusion is the primary treatment for haemoglobinopathies because it corrects the anaemia, suppresses abnormal erythropoiesis and replaces abnormal erythrocytes. This chapter discusses the haemoglobinopathies and RBC transfusion practices for their treatment.

α-Thalassemia

There are two α-globin genes (α-1 and α-2) on chromosome 16, so each person normally has four α-globin genes. Although more than 100 mutations of the α-globin protein have been identified [2], the severity of α-thalassemia typically correlates with the number of non-functional copies of the α-globin gene, which are a result of deletions or inactivating (non-deletional) point mutations. At equivalent levels of gene loss, non-deletional mutations, such as haemoglobin constant spring (CS), cause more severe phenotypes than deletions [3]. Diagnosis is made by the presence of Hb Barts (gamma globin tetramers γ_4) in infants or HbH (beta globin tetramers β_4) in those > 6 months of age. Both types of tetramers have very high oxygen affinity, preventing release of oxygen to tissues. HbH tetramers precipitate within red cells, forming insoluble inclusions (Heinz bodies) that damage the red cell membrane and cause variable levels of haemolytic anaemia. Ineffective erythropoiesis is less of an issue than in β- thalassaemia, although increased intestinal iron absorption can occur.

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Alpha-thalassaemias are classified into different subgroups depending on the number of alleles affected. When only one allele is affected (α-/αα) this is a benign and silent carrier state, also called 'alpha-thalassaemia minima'. Carriers may have mild hypochromia but are not anaemic or microcytic. When two alleles are affected, either in cis (--/αα) on the same chromosome or in trans (-α/-α) on different chromosomes, α-thalassaemia minor (also called α-thalassaemia trait) occurs. Patients have a mild microcytic hypochromic anaemia, but no other clinically obvious manifestations.

When three alleles are affected, either through deletion (--/α-) or also non-deletional mutations (--/ααND), thus resulting in only one functional α-globin gene, HbH disease occurs. HbH patients have marked clinical variability and non-deletional HbH, especially with the CS mutation, tends to be more severe than deletional HbH. Red cells in HbH disease are more prone to oxidative stress, so transfusion may be required with infections or oxidant drug exposure. Most patients with HbH do not require chronic transfusion, but those who become transfusion dependent tend to be symptomatic from early life.

Loss of all four alleles (--/--) causes hydrops fetalis, also called α-thalassaemia major, and is typically incompatible with live birth, with fetal death occurring between the late second trimester to within a few hours of birth. No α-globins are produced and the only haemoglobin present is Hb Barts, tetramers of gamma globin (γ_4), whose high oxygen affinity prohibits tissue oxygen delivery, resulting in high-output heart failure.

β-Thalassaemia

There is one β-globin gene on chromosome 11, so each person normally has two β-globin genes. β-thalassaemia is characterised by reduced (β^+) or absent (β^0) β-globin chain synthesis due to deletions or non-deletional mutations. There are over 200 mutations associated with β-thalassaemia; HbE is a common non-deletional β^+ mutation, with HbE thalassaemia having a wide range of clinical severity. Resulting unstable α-globin tetramers cause premature death of bone marrow erythroid precursors (ineffective erythropoiesis) and also peripheral haemolysis. Compensatory erythroid precursor expansion leads to bone marrow and extramedullary haematopoiesis, which then leads to reduced hepcidin levels, thus increasing intestinal iron absorption [4]. There are three main phenotypes: β-thalassaemia minor (also known as β-thalassaemia carrier or β-thalassaemia trait), β-thalassaemia intermedia and β-thalassemia major (also known as Cooley's anaemia or Mediterranean anaemia).

β-thalassaemia minor occurs in the carrier state; that is, only one β-globin gene is mutated (β^+/β, β^0/β). Globally, approximately 80–90 million people are reported to be carriers [5]. Patients have microcytosis, but are usually clinically asymptomatic, with only mild anaemia if any [5]. Anaemia becomes more marked during pregnancy and occasionally blood transfusion is necessary, although regular or frequent blood transfusions play no role.

β-thalassaemia intermedia occurs when both β-globin genes are mutated but some β chains are still formed (β^+/β^+ or $\beta^0\beta^+$). Clinical severity is highly variable, depending on the amount of excess unpaired γ-globin chains, although patients tend to have milder anaemia and present later than patients with β-thalassaemia major. Transfusions are typically given as needed, such as during infections, surgery, pregnancy or periods of rapid growth. However, some patients will become transfusion dependent, typically in the third or fourth decade of life [6].

β-thalassaemia major occurs when both alleles have mutations that produce minimal to no β-globin. The most common genotypes are $\beta^0\beta^0$ and β^0/HbE. Individuals have severe and lifelong anaemia that usually manifests around 6–12 months, when fetal Hb declines. Infants present with failure to thrive, pallor, irritability, jaundice and dark urine, and splenomegaly [5].

Red Blood Cell Transfusion in Thalassaemia

Individuals with β-thalassaemia major, as well as a subset of those with β-thalassaemia intermedia and HbH disease, will require chronic simple transfusion. The goals of RBC transfusion therapy in thalassaemia are to relieve symptoms and complications of anaemia and extramedullary haematopoiesis from ineffective erythropoiesis. Morbidities from anaemia include impaired growth and development and heart failure, while those from extramedullary haematopoiesis include bony expansion and deformities, osteopenia, hepatosplenomegaly and hypersplenism [5].

RBC transfusions should be administered regularly to prevent the Hb from dropping below a prespecified pretransfusion target; typically between 9.5 and 10.5 g/dL; this Hb level promotes normal growth and physical activity, regresses bone deformities and suppresses extramedullary haematopoiesis [7]. Higher targets (11–12 g/dL) may be indicated in patients with heart failure, fatigue, bony pain or other clinically significant extramedullary haematopoiesis. The posttransfusion haemoglobin should be approximately 12–13 g/dL and no higher than 15 g/dL to prevent hyperviscosity. For patient/family convenience, the time interval between transfusions, typically 3–4 weeks, should be kept as stable as possible, adjusting transfusion volumes as needed; typical transfusion volumes are ~15 mL/kg for children (range 10–20 mL/kg) and 2–4 units for older children and adults [7].

RBC transfusions will worsen the spontaneous iron overload caused by increased intestinal absorption. Iron overload should be managed with iron chelation therapy and not by lowering the pretransfusion Hb level [7]. Iron chelation can be initiated at the same time as chronic transfusion is started or after transfusion of approximately 20–25 RBCs, and should be guided by serum ferritin and liver and cardiac iron concentration levels. Once initiated, iron chelation requires close monitoring and follow-up.

Sickle Cell Disease

The HbS mutation, a single base substitution (A to T) in the β-globin gene leading to an amino acid change from glutamic acid to valine, is the most common Hb variant in the world. SCD is caused by homozygosity for the HbS mutation in the two β-globin genes (HbSS) or heterozygosity for the HbS mutation with another β-globin gene mutation, most commonly the HbC mutation or a β-thalassaemia mutation. HbS polymerises when deoxygenated, causing RBCs to eventually assume a sickle shape. Sickle RBCs are rigid and less deformable than normal RBCs, leading to haemolysis, and abnormally adhere to vascular endothelium and adherent white cells, leading to microvascular occlusion and reperfusion injury [8]. Downstream effects of haemolysis and microvascular occlusion include endothelial damage, vasoconstriction, systemic inflammation and tissue injury, resulting in severe pain episodes and acute and chronic end-organ damage, including but not limited to the spleen, kidneys, brain, lungs, heart and bone.

There are three main genotypes of SCD [9]. Sickle cell anaemia (HbSS) is the most prevalent (75%) and is typically associated with a severe course. SC disease (HbSC) is the second most prevalent genotype (18%) and usually has a less severe course, although certain complications, such as avascular necrosis and retinopathy, may be more prevalent than in SS disease. Sβ thalassaemia makes up about 6% of cases, with $S\beta^0$ being as severe as SS and $S\beta^+$ being milder. Moderators of SCD severity include β-globin gene haplotype, hereditary persistence of fetal haemoglobin ($\alpha_2\gamma_2$) and α-thalassaemia trait. Sickle trait (HbAS) is not regarded as SCD and is typically associated with a benign course, although certain complications, such as rhabdomyolysis and renal papillary necrosis, may occur.

Red Blood Cell Transfusion in Sickle Cell Disease

The goals of RBC transfusion therapy in SCD are to reduce the level of HbS-containing red cells and to maintain or increase haematocrit (Hct); increasing Hct may suppress endogenous erythropoiesis.

RBC transfusions may be given by simple transfusion or by exchange transfusion using a manual or automated method. For chronic transfusion, automated exchange is likely more effective than simple transfusion, and may be more effective than manual exchange, in minimising iron overload and achieving the target HbS level. With some apheresis devices, an option with chronic exchange is to perform isovolaemic haemodilution prior to the exchange, which, at given end Hct and HbS goals, may reduce the volume of red cells needed for the exchange or, for a given volume of red cells, may further lower the postprocedure HbS. Isovolaemic haemodilutions should be avoided in clinical scenarios where induction of further anaemia may be detrimental, such as recent cerebral ischaemic events; to reduce this risk, haemodilution should probably not decrease the haematocrit to less than 21% and/or more than 20% from baseline [10]. For patients with hypotension related to the haemodilution, 5% albumin may be considered instead of saline as the replacement fluid.

There are both acute and chronic indications for transfusion [11], which are summarised in Table 34.1. Some particular points of recommendation [10] are as follows:

1) For chronic transfusion indications, automated exchange is generally recommended over simple transfusion or manual exchange for optimal management of iron overload and HbS target.
2) For severe or rapidly progressive acute chest syndrome, automated or manual exchange, if available, is recommended over simple transfusion, due to the more rapid achievement of a low HbS level. It is also recommended in those who do not respond to initial treatment with simple transfusion, or with high pretransfusion Hb levels that preclude simple transfusion.
3) For preoperative transfusion, simple transfusion is suggested for patients with Hb levels of less than 9 g/dL, and post-transfusion haemoglobin levels should not exceed 11 g/dL; exchange transfusion should be considered for Hb levels of greater than 9–10 g/dL, aiming for post-transfusion haemoglobin levels of 10–11 g/dL; exchange transfusion should also be considered for patients undergoing very high-risk surgery (e.g. neurosurgery or cardiac surgery).
4) For pregnancy, prophylactic transfusions may be appropriate for selected women with high-risk obstetric features, such as twin pregnancies or other medical co-morbidities, or at high risk for SCD complications. Women who develop SCD-related complications during the current pregnancy, such as recurrent acute pain episodes or acute chest syndrome, should also be started on regular transfusion. A target Hb level > 7.0 g/dL and a peak HbS level (or HbS + HbC) < 50% are suggested.

Red Blood Cell Transfusion Requirements

Both patients with thalassaemia and those with SCD should receive leuco-reduced packed RBCs (pRBCs); this reduces febrile non-haemolytic transfusion reactions and human leucocyte antigen (HLA) alloimmunisation, which can be problematic in candidates for allogeneic bone marrow transplant. Patients with SCD should also receive units that are negative for HbS. A red cell antigen profile, by serological and/or molecular typing, should be sent at the earliest opportunity [10,12]; molecular typing is helpful to predict the red cell antigen phenotype in patients who have received transfusion within the prior three months and also aids in the identification of Rh variants that can

Table 34.1 Indications for transfusion in sickle cell disease.

Indication	Transfusion method recommendation
Acute	
Aplastic crisis	Simple
Acute sequestration (hepatic or splenic)	Simple (5 mL/kg) or exchange Exchange if hyperviscosity a concern and haemodynamically stable
Acute stroke (ischaemic or haemorrhagic)	Exchange preferred over simple
Acute chest syndrome	Simple for mild to moderate severity Exchange for severe, rapidly progressive, poorly responsive to simple transfusion, or high pretransfusion haemoglobin (Hb; i.e. ≥ 9 g/dL)
Acute multi-organ failure	Exchange
Acute intrahepatic cholestasis	Exchange
Mesenteric ischaemia	Exchange
Preoperative (moderate- to high-risk surgery using general anaesthesia)	Simple Exchange if high-risk surgery or with baseline Hb ≥ 9 g/dL
Chronic	
Primary or secondary stroke prophylaxis (overt or silent)	Exchange preferable to simple
Pregnancy	Simple for severe anaemia Simple or exchange for sickle complications or high obstetric, medical or fetal risk
Controversial	
Recurrent acute pain episodes	Simple or exchange after hydroxyurea failure
Recurrent acute chest syndrome	Simple or exchange after hydroxyurea failure
Recurrent splenic sequestration	Simple or exchange
Acute or recurrent priapism	Simple or exchange (target end haematocrit [Hct] < 30%)
Active leg ulcers	Simple or exchange
In trial	
Pulmonary hypertension and diastolic heart disease	Exchange transfusion

be clinically significant. At a minimum, red cells should be matched for Rh (C, E or C/c, E/e) and K antigens, the most common antigens implicated in alloimmunisation in these patient populations. If patients are alloimmunised to less immunogenic antigens such as Jk, Fy and S/s, extended matching should be considered. For all these reasons, communication of the diagnosis of thalassaemia or SCD to the blood bank is crucial. For patients who are heavily alloimmunised and have a pending procedure, appropriate time must be allocated to screen for any new alloantibodies and reserve the compatible units required to undergo the procedure safely. For automated red cell exchange, a pre- and post-procedure complete blood count and Hb fractionation should be obtained to optimise the procedure as needed. In patients whose extracorporeal volume with apheresis exceeds 10–15% of their total blood volume, an albumin prime is recommended.

Complications of Red Blood Cell Transfusion in Haemoglobinopathies

Patients with thalassemia and SCD, compared to other patient populations, are at increased risk for red cell alloimmunisation and delayed haemolytic transfusion reaction (DHTR). Given their frequency of red cell transfusion, they are also at risk of transfusion-related iron overload.

The prevalence of alloimmunisation in thalassaemia and SCD populations can be as high as 30–40% [13], compared to 3–5% in all transfused patients, even when corrected for cumulative transfusion burden [14]. Most antibodies are direct against the highly immunogenic Rh (CcEe) and Kell antigens; hence the recommendation for upfront Rh (C, E or C/c, E/e) and Kell matching. Reasons for this high rate of alloimmunisation are incompletely understood, although they include the high degree of minor antigen mismatches between donor and recipient [13,15,16], including Rh variant epitopes in either recipients or black donors, and transfusion during acute inflammatory events. One possible future solution is to provide more precise upfront antigen matching of units, including for Rh variants.

A DHTR is defined as a significant drop in Hb within 21 days post transfusion associated with one or more of the following: new red cell alloantibody, haemoglobinuria, accelerated HbS% increase with a concomitant fall in HbA post transfusion, relative reticulocytopenia or reticulocytosis from baseline, significant LDH rise from baseline, and exclusion of an alternative cause [10]. A DHTR to an alloantibody not identified prior to transfusion may occur with an anamnestic response, particularly with antibodies to the JK and FY system antigens, which can manifest only transiently. This is why it is crucial to contact other transfusion services from which patients may have previously received transfusion to determine whether any alloantibodies were identified earlier. In the scenario where there is no hyperhaemolysis (see below) or there is a risk for hyperhaemolysis and further transfusion is needed, implementing extended matching, which includes the anamnestically arisen alloantibodies, may be sufficient, in comparison to the scenario where hyperhaemolysis is present or a risk, where immunosuppression in addition to extended matching is recommended [10].

Hyperhaemolysis Syndrome

Hyperhaemolysis syndrome is a type of haemolytic transfusion reaction characterised by the rapid development of anaemia to a haemoglobin level lower than the pretransfusion level, accompanied by a rapid decline of the posttransfusion HbA level [10,17]. Clinical findings in hyperhaemolysis include fever, jaundice and pain, and laboratory findings include an elevated bilirubin, lactate dehydrogenase and typically a decrease in absolute reticulocyte count. New alloantibodies may or may not be present and a direct antiglobulin test (DAT) is negative in many cases. Although more common in SCD, hyperhaemolysis syndrome can occur in patients with thalassaemia also. One must be cognisant of the need to investigate recent transfusion history because, in SCD, hyperhaemolysis can mimic a vaso-occlusion pain episode. Recognition is critical, as additional transfusions should be avoided if possible. Erythropoietin with or without intravenous (IV) iron is also indicated, and possibly also intravenous immunoglobulin (IVIg) and steroids; in non-responders, eculizumab and tocilizumab are experimental treatments that can be tried [10,18]. If further transfusions are necessary, rituximab may be considered for potential prevention of additional alloantibody formation [17]. The exact mechanism for hyperhaemolysis syndrome has not been fully elucidated. but likely involves complement activation as well as macrophage activation [18,19].

Iron Overload

A unit of RBCs contains approximately 200 mg of iron. Since iron is not easily excreted from the body, simple transfusion, or red cell exchange where the end Hct is higher than the starting Hct, will eventually

lead to iron overload. Patients should be monitored for iron overload once they have received about 10 transfusions; monitoring should include serum ferritin, transferrin saturation and liver function testing. All chronically transfused patients should also have liver iron concentration assessed by magnetic resonance imaging (MRI; R2, R2* or Tw), unless they receive red cell exchange with a neutral or negative iron balance and have ferritin ≤ 1000 ng/mL. The same assessment method should be used over time. Thalassaemia patients should also have cardiac T2* MRI starting at age 10 and then at least every two years, depending on the degree of iron overload; cardiac iron can accumulate even with low liver iron concentration [20]. In patients with SCD, cardiac iron overload assessment is not recommended unless the patient has liver iron content > 15 mg/g dry weight for two years or more, a history of exceptionally elevated liver iron, evidence of end-organ damage resulting from transfusional iron overload, or evidence of cardiac dysfunction. The goal of iron chelation with both thalassaemia and SCD is to keep the ferritin level ≤ 1000 ng/mL or liver iron content ≤ 3 mg/g dry weight, and cardiac T2* > 20 msec.

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KEY POINTS

1) The goal of transfusions in patients with thalassaemia and sickle cell disease (SCD) is to treat the anaemia, suppress endogenous erythropoiesis, suppress extramedullary haematopoiesis (in the case of thalassaemia) and/or reduce HbS-containing red cells (in the case of SCD). The haematocrit goal, and in the case of SCD the HbS goal, must be appropriate for the clinical scenario.
2) As needed, patients with thalassaemia receive simple transfusion. In patients with SCD, automated red blood cell (RBC) exchange is generally recommended over simple transfusion or manual exchange for chronic transfusion management.
3) Patients with thalassaemia and SCD are at increased risk of delayed haemolytic transfusion reaction, so a red cell antigen profile should be obtained. Packed red blood cells should be leuco reduced, matched for Rh (C, E or C/c, E/e) and K antigens at a minimum and, in the case of SCD, HbS negative.
4) Management of hyperhaemolytic syndrome includes avoidance of transfusion if possible and erythropoietin; if transfusion is absolutely necessary, extended matched red cells are recommended along with immunosuppression.
5) Patients with thalassaemia and SCD receiving transfusion, especially chronic transfusion, must be monitored for iron overload in order to initiate iron chelation therapy to maintain ferritin levels ≤ 1000 ng/mL, liver iron content ≤ 3 mg/g dry weight and cardiac T2* > 20 msec.

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35 Transfusing Neonates and Infants

Marianne E. Yee[1,2] and Cassandra D. Josephson[1,2,3]

[1] *Aflac Cancer and Blood Disorders Center, Children's Healthcare of Atlanta, Atlanta, GA, USA*
[2] *Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA*
[3] *Department of Pathology and Laboratory Medicine, Center for Transfusion and Cellular Therapies, Emory University School of Medicine, Atlanta, GA, USA*

Neonates and infants have unique physiology and transfusion considerations, compared to older children and adults. The immature coagulation system, immature haematopoiesis, effects of maternal haematology and antibodies, as well as effects of prematurity and low birthweight are important considerations in the evaluation and treatment of neonates and infants with anaemia, thrombocytopenia or coagulopathy. Neonates are defined as ages 0–28 days, while infants are defined as ages > 28 days and < 1 year. Under the age of 4 months, naturally occurring anti-A and anti-B antibodies are not fully developed, and red blood cell (RBC) alloimmunisation is very rare. Prior to transfusion, ABO and RhD forward typing and an antibody screen are performed once in the neonate. If the initial antibody screen is negative, repeat antibody screens are not necessary until the age of 4 months. If the initial antibody screen is positive, due to maternal immunoglobulin (Ig)G, then pretransfusion ABO/D typing, antibody screen and compatibility testing should be repeated if > 72 hours from previous testing, as would be done for an infant ≥ 4 months of age, when considered immunologically competent from the blood bank and transfusion medicine perspective.

Transfusion support in neonates and infants is most commonly needed for those born prematurely. Preterm neonates and infants have immature haematopoiesis and thus decreased capacity for blood cell production, compared to term infants and children. Preterm is defined as birth before 37 weeks' gestational age, and subcategorised as extremely preterm (less than 28 weeks), very preterm (28–32 weeks) and moderate to late preterm (32–36 weeks). Birthweight is another important consideration when calculating transfusion doses as volume per kilogram of bodyweight. This is important for the interpretation of neonatal transfusion trials. Low birthweight is defined as < 2500 g, very low birthweight (VLBW) < 1500 g and extremely low birthweight (ELBW) < 1000 g.

Red Blood Cell Transfusion

RBCs are the most commonly transfused blood product for preterm neonates. Anaemia in preterm or sick infants is often exacerbated by iatrogenic blood loss due to phlebotomy for laboratory testing. Anaemia may be attributable to RBC loss (iatrogenic, haemorrhage or increased haemolysis) and/or to inadequate RBC production (inadequate erythropoietin

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[EPO] response, immature haematopoietic system, or rarely from thalassemia or bone marrow failure syndromes). Haemolysis may occur as a result of transplacental maternal antibodies or congenital haemolytic anaemias such as glucose-6-phosphate dehydrogenase (G6PD) deficiency and hereditary spherocytosis, necessitating RBC transfusion. Coagulopathies often associated with sepsis, necrotising enterocolitis (NEC), hypoxic ischaemic encephalopathy and haemorrhage also can contribute to anaemia among ill neonates and infants. The threshold for when to transfuse an infant or neonate typically depends on many factors, including age, gestational age, aetiology of anaemia and degree of other medical support needed.

In a term neonate at birth, the reference range for haemoglobin is 14–22 g/dL (average 18 g/dL) and haematocrit is 41–64% (average 52%) [1]. Over the first weeks of life, as haemoglobin synthesis transitions from fetal (HbF) to adult haemoglobin (HbA), haemoglobin declines to a physiologic nadir of approximately 11–12 g/dL by 10–12 weeks of life. This nadir is followed by a rise in EPO and increase in haemoglobin to normal levels. Preterm infants have a more exaggerated anaemia response, compounded by incomplete iron stores from the mother and immature erythropoiesis. The observed decline in haemoglobin occurs earlier and to a greater degree, with a nadir of approximately 8 g/dL for infants with birthweight 1000–1500 g and 7 g/dL in infants with birthweight < 1000 g. Delayed cord clamping (≥ 60 seconds) in preterm neonates has been shown in meta-analysis and a subsequent randomised controlled trial (RCT) to result in increased peak haematocrit in the first 7 days of life and decreased need for RBC transfusion, without difference in combined outcomes of mortality or major morbidity [2].

Studies of EPO-stimulating agents in preterm infants have shown conflicting impact on adverse outcomes. A recent RCT, Preterm Erythropoietin Neuroprotection Trial (PENUT), showed that EPO treatment did not decrease death or severe neurocognitive impairment by age 2 years, nor was there a difference in other adverse events including retinopathy of prematurity (ROP), intracranial haemorrhage (ICH), NEC, bronchopulmonary dysplasia (BPD) or sepsis, compared to placebo. However, infants who received EPO had a lower rate of transfusion, higher haematocrit levels, and were more likely to remain transfusion free by 12 weeks of life [3,4].

Haemolytic disease of the fetus and newborn (HDFN) occurs as result of maternal alloantibodies to fetal RBC antigens. ABO antibodies may cause haemolytic disease in a neonate, but do not affect the fetus. Severe HDFN is most often caused by anti-D, anti-c or anti-K. With routine use of Rh immune globulin (RhIg) during pregnancy in RhD-negative women, anti-D is now a less common cause of HDFN [5]. If severe hyperbilirubinaemia occurs due to HDFN (total bilirubin ≥ 25 mg/dL, or lower threshold based on neonatal age), exchange transfusion with reconstituted whole blood may be necessary to prevent kernicterus. Donor RBCs that are either O negative or ABO/Rh type specific and lack the implicated RBC antigen should be reconstituted with AB or compatible plasma to a final haematocrit of 40–60%. A double-volume exchange is recommended, which removes > 85% of infant RBCs and approximately 50% of plasma bilirubin. Manual exchange is performed by transfusing small increments of whole blood, while removing aliquots of the neonate's blood (e.g. 5 mL/kg or 5% total blood volume).

Red Blood Cell Transfusion Thresholds in Infants and Neonates

The majority of preterm neonates born under 30 weeks' gestational age require at least one RBC transfusion [6]. Term infants are more likely to need RBC transfusion related to surgery or extracorporeal membrane oxygenation (ECMO). Multiple studies have compared restrictive (lower) versus liberal (higher) transfusion thresholds for preterm infants, with conflicting results.

In these studies, haemoglobin level triggering transfusion in these studies varies based on age and severity of respiratory support and/or illness.

The first RCT of liberal versus restrictive transfusion thresholds found that those in the restrictive transfusion group had more major adverse events, including grade 4 intraventricular haemorrhage (IVH) and apnoea of prematurity. Subsequently, the Premature Infants in Need of Transfusion (PINT) study of 456 preterm, ELBW infants showed no difference in the composite primary outcome of death or major morbidity (severe ROP, BPD or brain injury) between those randomised to lower or higher transfusion thresholds [7]. Infants enrolled in the PINT trial were followed to a corrected age of 18–21 months, showing no significant difference in the composite primary outcome of death or cerebral palsy, cognitive delay, vision or hearing impairment; however, *post hoc* analysis of milder cognitive delay measures (Bayley Scales of Infant Development-II Mental Developmental Index [MDI] score < 85) suggested that infants who had received transfusion in the higher-threshold group had lower rates of cognitive delay [8].

Two recently completed trials examined the outcomes of both death and neurological disability at approximately 2 years' corrected age. The Effect of Transfusion Thresholds on Neurocognitive Outcomes (ETTNO) trial, which randomised 1013 ELBW infants to a liberal transfusion strategy (haematocrit 28–35% for non-critical, 34–41% for critical infants) versus a restrictive (haematocrit 21–28% for non-critical, 27–34% for critical infants) strategies found no difference in death or neurological outcome (MDI score < 70) at 24 months' corrected age. Rates of NEC, BPD and ROP were similar in the two treatment arms [9]. The multicentre Transfusion of Prematures (TOP) trial, which randomised 1824 ELBW infants to a high transfusion threshold (haemoglobin 10.0–13.0 g/dL) versus a low threshold (haemoglobin 7.0–11.0 g/dL), used a milder definition of cognitive deficit (MDI score < 85) than ETTNO, yet still found no difference in death or neurological outcome at 22–26 months' corrected age for higher versus lower thresholds [6].

While these trials provide valuable information on the impact of transfusion threshold in VLBW neonates, limitations of these results should be noted. First, although the restrictive (low) transfusion threshold did not demonstrate a difference in gross neurological impairment, the studies were not designed to assess for milder cognitive or intellectual disabilities that might not be identified at age 2 years. Second, although these trials defined permissible lower limits of haemoglobin by week of life and need for respiratory support, actual haemoglobin levels in the infants (both pre transfusion and overall) were higher than the study's lower limits. In the TOP trial, although the haemoglobin threshold was set as low as 7 g/dL, the mean pretransfusion haemoglobin nadir did not drop below 8 g/dL in the low-threshold arm. The average haemoglobin throughout the study was > 10 g/dL in the low-threshold arm and > 11 g/dL in the high-threshold arm [6]. Conclusions about the short-term or long-term effects of severe permissive anaemia cannot be derived from these studies, and the use of lower thresholds must be approached with caution. Lastly, the study results do not inform decisions for transfusion during medically unstable events or for infants with cardiopulmonary disease. Table 35.1 shows common guidance for transfusion thresholds in different clinical situations, although no definitive studies exist regarding thresholds in many scenarios.

Adverse Outcomes of Anaemia and Transfusion in Neonates

Adverse transfusion events are more common among infants under 12 months compared to older children and adults [10]. The potential relationships of anaemia and transfusion to complications of prematurity, particularly NEC, are another area of focus. Early retrospective studies and meta-analysis

Table 35.1 Guidelines for small-volume (10–15 mL/kg) red blood cell (RBC) transfusion in neonates and infants.

Situation	RBC transfusion threshold (haematocrit)
Anaemia in first 24 hours of life	30–35%
Clinically stable preterm neonate	25–30%
Clinically stable term neonate	20–25%
Clinically unstable, major surgery or bleeding neonate	30–35%
Moderate cardiopulmonary disease	30–35%
Severe cardiopulmonary disease or extracorporeal membrane oxygenation (ECMO)	35–45%

demonstrated association between RBC transfusion and NEC; however, later meta-analysis of observational studies showed no significant association [11]. A prospective, multicentre cohort study of 598 VLBW infants followed from birth through to 90 days or discharge examined both RBC transfusion and severe anaemia (Hb < 8 g/dL) as predictors of NEC. Although the unadjusted incidence of NEC was higher among infants with RBC transfusion exposures, multivariable analysis showed that the rate of NEC was significantly increased among infants with severe anaemia in a given week, but not for infants with RBC transfusion [12]. This study suggests a role for severe anaemia rather than transfusion in the pathogenesis of neonatal adverse outcomes, and demonstrates the need for more sophisticated studies of tissue oxygenation and transfusion in the optimal support of neonates.

Selection of Red Blood Cell Products for Neonates

During pregnancy, transplacental transfer of maternal IgG occurs, which may include anti-A, anti-B, anti-A,B or other minor RBC antigen antibodies. Additionally, with maternal–fetal haemorrhage, IgM may be transferred. Therefore, RBC units for neonates must be compatible with both the neonatal blood type and possible maternal antibodies. Many institutions transfuse only group O RBCs to neonates for this reason. Alternatively, neonates may receive ABO-specific transfusion if plasma or serum testing of the neonate or mother does not demonstrate IgG or IgM directed against the neonate's RBC [13].

Most RBC transfusions to neonates and infants are small volume (10–15 mg/kg) in extended-storage media (additive solution AS-1, AS-3, AS-5) or citrate-phosphate-dextrose-adenine (CPDA) solution. The concentration of additives in RBC units is generally safe up to transfusion volumes of 20 mL/kg (small-volume transfusion), but there are little data for safety in neonates during exchange transfusion, ECMO or cardiopulmonary bypass, which requires > 20 mL/kg of RBCs (large-volume transfusion). Concerns about higher concentrations of adenine and mannitol (AS-1 and AS-5) include potential nephrotoxicity and diuresis exist with additive units, thus non-additive (CPD) units may be used for neonates for small- and large-volume transfusions.

Multiple transfusions may be derived from a single dedicated unit for its storage duration, to reduce donor exposures. The Age of Red Blood Cells in Premature Infants (ARIPI) trial, which randomised VLBW infants to receive units stored for 7 days or less (mean 5.1±2.0 days) versus standard issue (mean age 14.6±8.3 days) found no difference in death, NEC, ROP, BPD, IVH or nosocomial infection between the groups [14]. A critique of the study is that very few infants received RBC aged ≥ 14 days, thus outcomes may not be generalisable to the use of older units [15].

Platelet Transfusion

Thrombocytopenia (platelet count < 150 000/μL) is uncommon in term infants, but is more likely among preterm infants, affecting the majority of ELBW neonates. Thrombocytopenia may be caused by increased platelet destruction, decreased production or both. Thrombocytopenia at birth is likely related to maternal conditions (hypertension, placental insufficiency, maternal antibodies or congenital infection), while later-onset thrombocytopenia is common with sepsis, NEC and consumptive coagulopathy. Rare inherited bone marrow failure syndromes and transient abnormal myelopoiesis (TAM) in trisomy 21 should be considered as causes of hypoproliferative thrombocytopenia.

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a common cause of thrombocytopenia in an otherwise healthy term or preterm neonate. FNAIT is a result of maternal alloimmunisation to paternally derived human leucocyte antigen (HLA) or human platelet antigen (HPA), with transplacental transmission of maternal antibodies to the fetus bearing those antigens. Infants with FNAIT are at particular risk for ICH (prenatally or postnatally), often necessitating platelet transfusion for severe thrombocytopenia. The most common antigen associated with FNAIT among women of European ancestry is HPA-1a, occurring in approximately 80% of cases. Of the 2% of women who are HPA-1a negative, the histocompatibility antigen DRB3*0101 is the major risk factor for developing alloimmunisation to HPA-1a [16]. Other antigens implicated in NAIT include HPA-1b, HPA-3a, HPA-5b, HPA-9b and HPA-15b. Among women of African ancestry, alloimmunisation to HPA-2 and HPA-5 is more common, while in women of Asian ancestry, alloimmunisation to HPA-4b is the predominant cause of FNAIT [17]. Additionally, maternal autoantibodies to platelets, as seen in immune thrombocytopenic purpura (ITP), may cause neonatal thrombocytopenia, although the frequency of severe thrombocytopenia (platelet count < 50 000/μL) and ICH is less than with FNAIT [18].

Neonates with very severe thrombocytopenia (< 30 000/μL) due to FNAIT should receive immediate treatment, given the high risk of serious bleeding. Transfusion with random donor platelets, even if positive for the implicated antigen, may produce a transient rise in platelet count, thus transfusion should *not* be delayed while seeking antigen-negative platelets from either the mother or another donor. Intravenous immunoglobulin (IvIg) 0.4–1.0 g/kg/day for 2–5 days may also increase the platelet count. HPA-compatible platelets may be obtained from HPA-typed donors or from maternal platelet pheresis. Maternal platelets must be washed to remove the causative alloantibody, and require irradiation as a precaution against transfusion-associated graft-versus-host disease (TA-GVHD) [16].

Platelet Transfusion Thresholds in Infants and Neonates

The majority of hospitalised neonates with severe thrombocytopenia will have bleeding; however, the platelet count does not have a direct correlation with degree of bleeding. An RCT of preterm, thrombocytopenic infants in 1993 demonstrated no difference in the rate of ICH among infants transfused at a threshold of 50 000 versus 150 000/μL, although those transfused to maintain the higher platelet count required less RBC and fresh frozen plasma (FFP) transfusion. In 2019, the Platelet Transfusion Thresholds in Premature Neonates (PlaNet-2) RCT, which compared thresholds of 25 000 versus 50 000/μL in 660 preterm infants with severe thrombocytopenia, unexpectedly found that the higher-threshold group had a higher frequency of the outcome of major bleeding episode or death as well as bronchopulmonary dysplasia [19]. Table 35.2 shows recommended platelet transfusion threshold ranges for different clinical scenarios.

Table 35.2 Guidelines for platelet transfusion in neonates and infants.

Situation	Platelet transfusion threshold
Clinically stable term neonate	20 000–25 000/μL
Clinically stable preterm neonate	First week of life: 50 000–75 000/μL After first week of life: 25 000/μL
Clinically unstable or bleeding neonate	50 000–100 000/μL
Need for invasive procedure	50 000/μL
Neonatal alloimmune thrombocytopenia (NAIT)	30 000–50 000/μL
Extracorporeal membrane oxygenation (ECMO)	50 000–100 000/μL

Pathogen-Inactivated Platelet and Plasma Transfusion

Pathogen reduction (PR) or pathogen inactivation (PI) of platelet products has been adopted in many countries to prevent bacterial, viral and parasitic transfusion-transmitted infection, as well as inactivation of residual donor white blood cells (WBCs). PR/PI processes utilise either photochemical activation (e.g. psoralen-derivative compounds) or solvent/detergent (S/D) treatment. The INTERCEPT Blood System uses amotosalen to intercalate within DNA, followed by ultraviolet A (UVA) exposure, preventing DNA transcription. Theoretical concerns have been raised about the effects of psoralen and UVA exposure on paediatric stem cells. Additionally, if neurocognitive effects of these compounds are not recognised, neonates and infants are at highest risk for long-term sequelae, given their small body mass and active cognitive development [20]. While INTERCEPT products are not contraindicated in children, there are few published data on their use in neonates.

Cellular Product Modifications for Neonates and Infants

Leuco reduction is important for all neonates and infants, for the prevention of cytomegalovirus (CMV) transmission, febrile non-haemolytic transfusion reactions and HLA alloimmunisation. Because premature infants are at risk for severe CMV infection, risk-reduction strategies such as selecting CMV-seronegative and leuco-reduced blood for those with birthweights ≤ 1500 g should be considered if possible. If CMV-seronegative cellular products are not available, CMV risk reduction by leuco reduction is a safe, alternative option. All other neonates and infants may receive leuco-reduced products to prevent transfusion transmission of CMV [21,22].

Irradiation of cellular products prevents TA-GVHD in patients with immunodeficiency or underdeveloped immune systems. Practices regarding irradiation of cellular products transfused to infants may vary among institutions. All infants with known or suspected immunodeficiencies, receiving bone marrow transplantation or receiving related, directed donor transfusion require irradiation of cellular products. Some institutions elect to irradiate cellular products transfused to all neonates and infants, given the potential for an undiagnosed congenital immunodeficiency in this age range. Recently the British Society for Haematology Guidelines Transfusion Taskforce recommended that routine irradiation of RBC or platelet transfusions to term or preterm infants is not required unless they had received intrauterine transfusion (requiring irradiated components until 6 months after the expected delivery date of

40 weeks' gestation) or if receiving exchange blood transfusion [23]. This recommendation is expert opinion based and supportive data are still lacking, hence it is not an accepted practice worldwide.

Non-cellular Plasma Products

Neonates and infants have immature haemostatic systems, with many coagulation factors at birth being approximately half adult levels. This can lead to challenges in the interpretation of coagulation testing and determination of when plasma transfusion is indicated. Pathological coagulopathy may occur with sepsis, disseminated intravascular coagulopathy (DIC), liver disease, vitamin K deficiency and dilution from massive transfusion, thus necessitating plasma transfusion. Most congenital factor deficiencies (factor VII, VIII, IX, X, XIII) can be treated with specific factor concentrates; however, some rare factor deficiencies (factor V, XI) may not have available concentrates, or available concentrates may not be readily available for rare deficiencies in emergent situations. For replacement of coagulation factors, initial plasma therapy should be 10–15 mL/kg, which raises factor levels by approximately 30%. Plasma is also required when reconstituting whole blood for neonatal exchange transfusion, cardiopulmonary bypass and ECMO [24].

KEY POINTS

Red blood cells

1) In preterm, extremely low birthweight infants, although the TOP and ETTNO trials showed non-inferiority of a lower versus higher red blood cell (RBC) transfusion thresholds, the lowest thresholds (haemoglobin < 7 g/dL) were not adequately evaluated.
2) Severe anaemia (haemoglobin ≤ 8 g/dL), but not RBC transfusion, is associated with the development of necrotising enterocolitis in preterm infants.
3) High-dose erythropoietin therapy does not reduce neurodevelopmental impairment or death, but does reduce RBC transfusion in preterm infants.
4) All extended-storage media and standard citrate-phosphate-dextrose-adenine (CPDA-1) solutions for RBC units are safe for infants for low-volume transfusion (≤ 20 mL/kg).
5) The most common cause of haemolytic disease of the fetus and newborn is ABO incompatibility between mother and fetus. If total bilirubin exceeds 25 mg/dL (or lower threshold, based on neonatal age, gestational age and rate of rise), double-volume, manual exchange with reconstituted whole blood is recommended.

Platelets

6) In preterm infants with severe thrombocytopenia (platelets < 50 000/μL), the PlaNet-2 trial showed a higher rate of death and serious bleeding with a higher platelet transfusion threshold (50 000/μL vs. 25 000/μL), but did not study infants within the first week of life, thus does not support a lower threshold for neonates < 1 week old.
7) There is a lack of strong evidence to support the use of INTERCEPT pathogen-reduced platelet products in infants and neonates.
8) If maternal platelets are used to treat fetal and neonatal alloimmune thrombocytopenia, the units must be washed to remove the causative antibody and prevent further immune-mediated destruction of the neonatal platelets, and must be irradiated to prevent transfusion-associated graft-versus-host disease.

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36 Immunodeficiency and Immunoglobulin Therapy

Siraj A. Misbah

Oxford University Hospitals, University of Oxford, Oxford, UK

The increasing awareness of immunodeficiency and the rapid pace of genetic discovery have helped to ensure that immunodeficiency disorders are no longer viewed as arcane rarities by both clinical immunologists and non-immunologists. In haematology, alongside the major changes in practice that have been driven by advances in fundamental immunology [1], haematologists are also likely to encounter patients with primary immunodeficiency disease due to the frequency of haematological complications associated with this group of disorders. Given that most haematologists will be familiar with the consequences of secondary immunodeficiency, either iatrogenic or associated with lymphoproliferative disease, this chapter will focus principally on primary immunodeficiency disorders (PID), followed by a separate section on immunoglobulin therapy.

Primary Immunodeficiency Disorders

Many PIDs associated with single gene mutations have been aptly called experiments of nature, in view of the unique insights that these diseases have provided in unravelling complex immunological functions. Currently, the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Committee on Primary Immunodeficiency Diseases recognises over 400 primary immunodeficiencies for which the underlying molecular basis has been elucidated [2]. As the genetic basis of old and new immunodeficiency disorders is unravelled, it has become clear that the same gene mutation may result in different phenotypes. In investigating and managing patients with PID, it is important to bear in mind this concept of genetic heterogeneity accompanied by equally significant clinical and immunological heterogeneity. For example, the same mutation in the gene encoding the Wiskott–Aldrich syndrome protein (WASP) may result in either full-blown Wiskott–Aldrich syndrome characterised by thrombocytopenia, infections and autoimmunity, or a limited phenotype of X-linked thrombocytopenia [3]. Similarly, mutations in recombination-activating genes (RAG) may present with a wide range of distinct immunological phenotypes beyond severe combined immunodeficiency. Conversely, a distinct immunophenotypic syndrome may be associated with a wide range of mutations in different genes. Such examples have focused attention on the role of epigenetic changes in influencing disease phenotype, in addition to highlighting the limitations of using immunophenotypic patterns as a guide to underlying molecular defects.

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Although PID can affect any part of the immune system, in practice patients with predominant defects of B-cell function and combined B- and T-cell defects constitute the bulk of a clinical immunologist's workload. The immunopathogenesis of antibody deficiency disorders and combined B- and T-lymphocyte deficiency is best understood within the context of B- and T-lymphocyte development. The schematic diagrams set out in Figures 36.1 and 36.2 summarise the major events in B- and T-cell development and the points at which developmental arrest leads to immunodeficiency. The delineation of new immunodeficiencies using whole exome and next-generation sequencing has led to calls for a revised classification of PID, which integrates clinical, immunological and genetic phenotypes [4].

Predominant B-Cell Deficiency Disorders

Common Variable Immunodeficiency

Of the 46 antibody deficiency disorders currently recognised, common variable immunodeficiency (CVID) is the most common acquired PID that is likely to be encountered by haematologists. As its name implies, CVID is characterised by a severe reduction in at least two serum immunoglobulin isotypes associated with low or normal B-cell numbers. In contrast, antibody deficiency disorder associated with severe reduction of all serum immunoglobulin isotypes with absent circulating B cells is a feature of diseases associated with mutations that interrupt B-cell development (see Figure 36.2).

The term CVID embraces a heterogeneous group of disorders, all of which are characterised by late-onset hypogammaglobulinaemia as the unifying theme [5]. The most

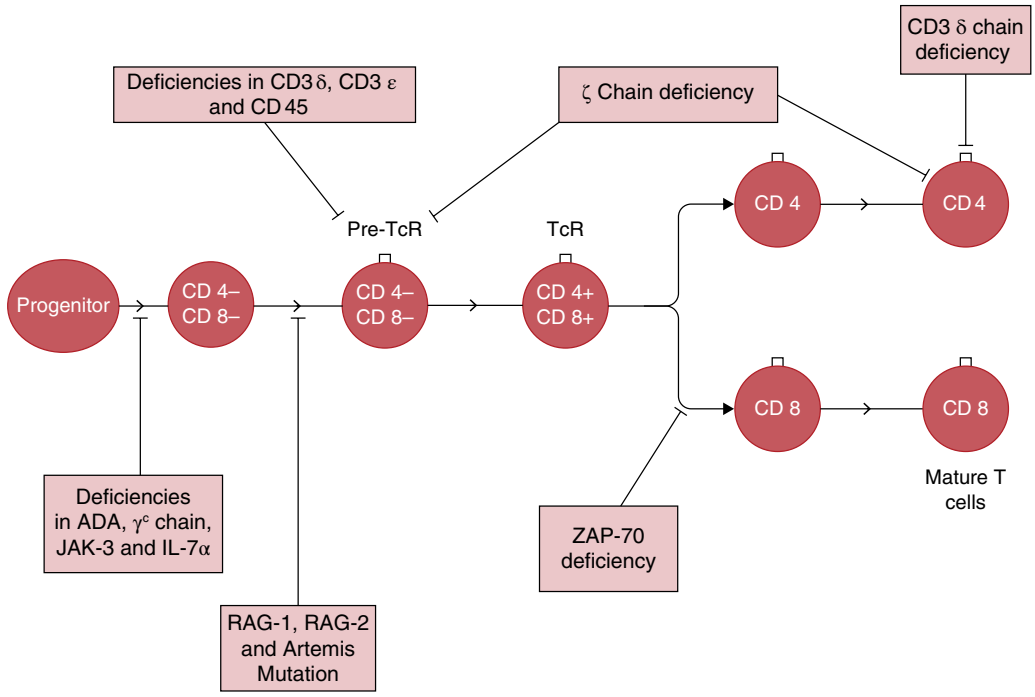

Figure 36.1 Major events in T-cell development and sites of mutations leading to immunodeficiencies. T-cell development in the thymus from a progenitor cell proceeds sequentially from a double-negative state (CD4–, CD8–) to mature T cells expressing either CD4 or CD8. Stages of T-cell differentiation associated with mutations and deficiencies of proteins are depicted in boxes. ADA, adenosine deaminase; JAK3, Janus kinase 3; RAG, recombination-activating gene; ZAP-70, zeta-chain-associated protein of 70 kd. *Source:* Adapted from Rudd CE. NEJM 2006;354:1874.

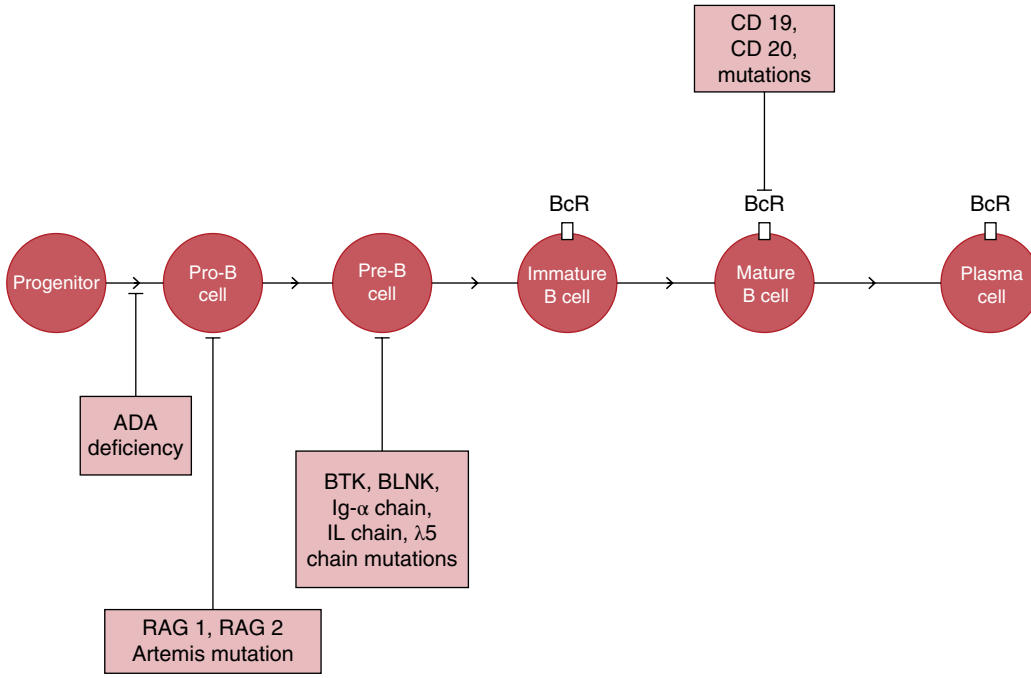

Figure 36.2 Major events in B-cell development and sites of mutations leading to immunodeficiencies. B-cell development in the bone marrow proceeds from a progenitor cell sequentially to plasma cells in the periphery. Stages of B-cell differentiation associated with mutations and deficiencies of proteins are depicted in boxes. ADA, adenosine deaminase; BLNK, mutated B-cell-linked protein; BTK, Bruton tyrosine kinase; RAG, recombination-activating gene. *Source:* Adapted from Rudd CE. NEJM 2006;354:1874.

common infective manifestation of antibody deficiency is recurrent infection with encapsulated bacteria, particularly *Streptococcus pneumoniae* and to a lesser extent unencapsulated *Haemophilus influenzae*. Many patients develop frank bronchiectasis as a consequence of recurrent chest infections. Despite their inability to mount effective antibody responses to exogenous pathogens, many patients with CVID mount paradoxical immune responses to self antigens, leading to autoimmune disease. In a haematological context, the most frequent of these autoimmune complications are immune thrombocytopenic purpura (ITP) and autoimmune haemolytic anaemia.

A whole host of other organ-specific and systemic autoimmune diseases may also occur, ranging from Addison's disease to systemic lupus erythematosus. Non-infective complications associated with CVID include a curious predisposition to granulomatous disease, lymphoid interstitial pneumonitis and a 100-fold increase in the risk of lymphoma. Although the latter may occasionally be driven by Epstein–Barr virus (EBV), in the majority of cases no underlying infection is evident, raising the possibility that lymphoproliferative disease in these patients is a manifestation of defective immunoregulation.

Despite the inability of B cells in CVID to produce antibodies, recovery of antibody production has been documented following infection with hepatitis C virus (HCV) or human immunodeficiency virus (HIV) [6]. This observation supports the concept that defective immunoregulation is contributing to poor B-cell function in these patients.

Given the range of infective and non-infective complications associated with CVID, many attempts have been made to produce a clinically useful disease classification

based on immunological indices. Recent evidence suggests that a deficiency of switched immunoglobulin (Ig)M^- IgD^- $CD27^+$ memory B cells may correlate with the development of bronchiectasis, autoimmunity and reactive splenomegaly in CVID. The molecular basis for some of the diseases previously included under the umbrella of CVID has recently been elucidated by the detection of mutations in a number of genes associated with B-cell function (Box 36.1). This list is not exhaustive and represents a representative sample from over 60 disease-causing gene mutations identified in CVID. Based on the population studied, between 31% and 54% of patients with CVID have been shown to have pathogenic mutations, with higher rates being recorded in populations with high rates of consanguinity [7]. In addition to the molecular defects listed in Box 36.1, there are rare patients with mutations in certain X-linked genes (Bruton tyrosine kinase, CD40 ligand and signalling lymphocyte activation-associated protein), who may present with a clinical phenotype resembling CVID.

The management of CVID revolves around regular immunoglobulin replacement optimised to ensure a trough IgG level well within the normal range for effective prophylaxis against bacterial infections. Evidence from a longitudinal study of infection outcomes in 90 patients with CVID followed up over 20 years suggests that the dose of immunoglobulin required to reduce breakthrough infections is individual to a particular patient [8]. Achievement of this goal is therefore likely to be associated with a wide range of trough IgG levels. Early diagnosis and therapeutic intervention with immunoglobulin therapy significantly minimise the risk of permanent bronchiectatic lung damage.

X-Linked Agammaglobulinaemia

X-linked agammaglobulinaemia (XLA) was one of the earliest primary immunodeficiencies to be clinically characterised, in the 1950s. Its molecular basis was only elucidated in the 1990s with the discovery of mutations in a protein tyrosine kinase gene, named Bruton tyrosine kinase (Btk).

The Btk gene is located on the long arm of the X chromosome and encodes for a cytoplasmic tyrosine kinase, which is essential for B-cell signal transduction. Btk mutations are associated with B-cell developmental arrest in the bone marrow. The consequent disappearance of circulating B cells in association with severe panhypogammaglobulinaemia and poorly developed lymphoid tissue

Box 36.1 Known molecular defects that present with a common variable immunodeficiency (CVID)-like clinical picture.

- Inducible co-stimulatory receptor (ICOS) deficiency.
- CD19 deficiency.
- Mutations in the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) receptor.
- Mutations in the receptor for B-cell-activating factor of the tumour necrosis factor (TNF) family (BAFF).
- Mutations in cytotoxic T-lymphocyte-associated protein (CTLA-4).
- Mutations in nuclear factor–kappa B2 gene (NFKB2).
- Mutations in LPS-responsive vesicle trafficking beach and anchor enhancing (LRBA) gene.
- Mutations in phosphatidyl-3-kinase C δ (PIK3CD) gene.
- CD20 deficiency.
- CD21 deficiency.
- CD81 deficiency.

constitute the cardinal immunological features of XLA. Over 400 different mutations in the Btk gene have been recorded to date, but there are no significant correlations between genotype and clinical phenotype. The essential role of Btk in B-cell receptor signal transduction, as exemplified by B-cell failure in XLA, has been exploited by the development of Btk inhibitors (ibrutinib) for the treatment of B-cell lymphomas.

Most boys with XLA present with a history of recurrent sinopulmonary infections on a background of pan-hypogammaglobulinaemia after the age of 6 months, once the protective effect of transplacentally acquired maternal IgG has waned. As with CVID, delayed diagnosis of XLA and consequent failure to institute adequate immunoglobulin replacement are associated with a high risk of bronchiectasis [9].

In keeping with the absence of a T-cell defect in XLA, infection with intracellular pathogens is generally not a problem. The major exception to this rule is the predisposition to chronic enteroviral infections, including echovirus meningoencephalitis and vaccine-induced poliomyelitis. A clinical phenotype identical to XLA may be caused by mutations in the μ-immunoglobulin heavy chain gene and other components of the B-cell receptor [10].

Severe Combined Immunodeficiency

Severe combined immunodeficiency (SCID) refers to a group of genetically determined disorders characterised by arrested T-cell development accompanied by impaired B-cell function [11]. The incidence of SCID is estimated to be between 1 : 50 000 and 1 : 100 000 live births.

Babies with SCID present with recurrent infections associated with lymphopenia. Among the range of pathogens responsible for infection in SCID, *Pneumocystis jiroveci (carinii)*, *Aspergillus* species and cytomegalovirus (CMV) predominate, in keeping with the profound T-cell deficiency seen in these babies.

To date, at least 18 distinct molecular defects that cause the SCID phenotype have been identified, with 8 of the more common defects being listed in Table 36.1. While lymphopenia is characteristic of all forms of SCID, the circulating lymphocyte surface marker profile (see Box 36.1) provides a useful clue as to the underlying genetic defect. For example, deficiency of adenosine deaminase, a key purine enzyme, results in severe lymphopenia affecting T, B and natural killer (NK) cells, leading to its characterisation as T–B–NK–SCID. Based on an analysis of 172 babies with SCID transplanted at a single centre over a 30-year period, three major diagnostic clues have emerged as markers of all

Table 36.1 Classification of severe combined immunodeficiency.

Affected gene	Inheritance	Circulating lymphocyte phenotype
Adenosine deaminase (ADA)	AR	T– B– NK–
Common cytokine γ-chain (γc)	X-linked	T– B+ NK–
Jak-3	AR	T– B+ NK–
IL-7α	AR	T– B+ NK+
Recombination-activating gene 1, 2 (RAG1/RAG2)	AR	T– B– NK+
Artemis	AR	T–B–NK+
CD3 δ, ζ, ε	AR	T– B+ NK+
CD45	AR	T– B+ NK+

AR, autosomal recessive.

molecular forms of SCID: a positive family history was noted in 37%, lymphopenia in 88% and an absent thymic shadow in 92% [12].

Given the profound impairment in T-cell immunity, babies with SCID are at risk of iatrogenic disease with live vaccines and transfusion-associated graft-versus-host disease. For these reasons, immunisation with live vaccines should be regarded as absolutely contraindicated in these babies. Equally, any baby with SCID should only receive irradiated and CMV-seronegative blood.

The severity of disease and the urgency with which curative haemopoietic stem cell transplantation (HSCT) should be undertaken have led SCID to be regarded as a paediatric emergency. The results of HSCT have improved significantly with early diagnosis and aggressive management of infections and nutritional problems seen in these babies at the time of diagnosis. At present, HSCT from an HLA-matched sibling donor offers an 80% chance of cure, while a fully HLA-matched unrelated transplant offers a 70% chance of cure (Figure 36.3). These results from Europe have recently been confirmed by longitudinal outcome data in 240 infants with SCID transplanted during a 10-year period (2000–09) in the USA [13]. Neonatal screening for SCID using polymerase chain reaction–based analysis of T-cell receptor excision circles (TRECs – a measure of thymic T-cell output) on Guthrie card blood samples has been required in all states in the USA since 2018 [14].

In view of the single gene defects underlying SCID, gene therapy is an attractive option. While offering great promise, the results of gene therapy to date have been mixed. Gene therapy has been successful in some children with adenosine deaminase

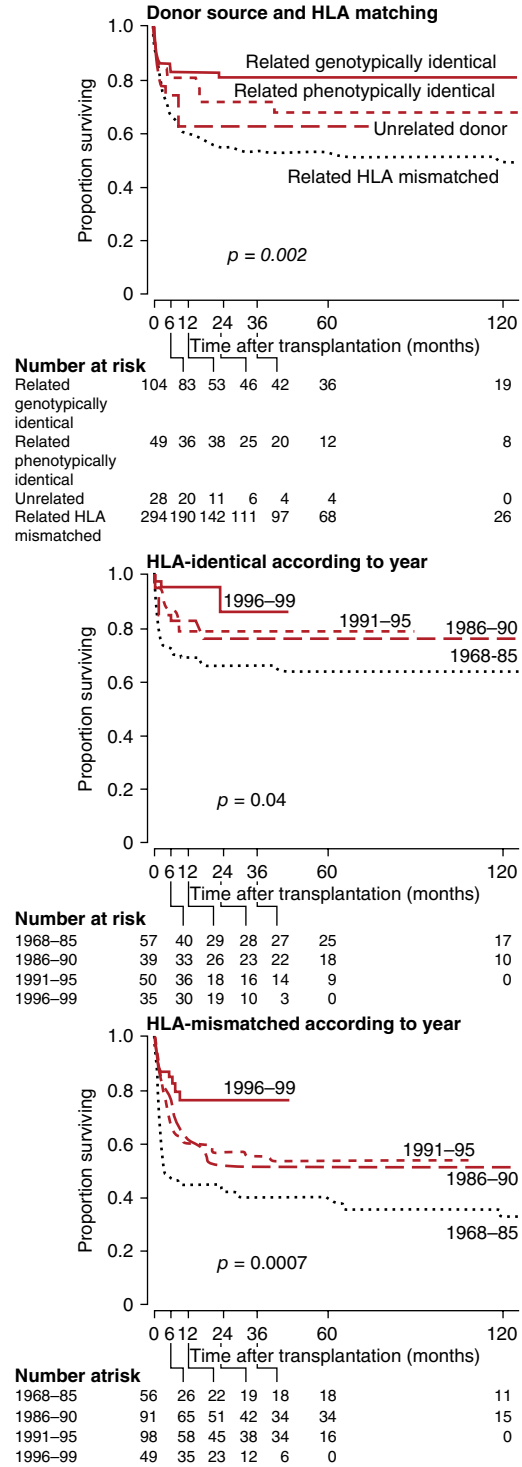

Figure 36.3 Cumulative probability of survival in severe combined immunodeficiency (SCID) patients, according to donor source (related or unrelated donor) and human leucocyte antigen (HLA) matching, and year of transplantation. *Source:* Antoine C et al. Lancet 2003;**361**:556. Reproduced with permission of Elsevier.

(ADA) or common cytokine γ-chain deficiency, with evidence of T-, B- and NK-cell reconstitution in the former and T- and NK-cell reconstitution in the latter. However, the occurrence of insertional mutagenesis leading to T-cell lymphoproliferative disease in some children with common γ-chain SCID is an important reminder of the obstacles associated with this ground-breaking therapy [15]. This risk has prompted the development of a new generation of self-inactivating and lentiviral vectors, which have been used in common γ- chain SCID. To date, there have been no reports of insertional mutagenesis [16].

Investigation of Suspected Immunodeficiency

Although a few patients may have distinctive clues on examination pointing towards a PID, most patients have no physical signs that would specifically point to an immunodeficiency disorder. Conversely, it follows that a normal physical examination does not exclude immunodeficiency disease.

Immunodeficiency should be included in the differential diagnosis of any patient with severe, prolonged or recurrent infection with common pathogens, or even a single episode of infection with an unusual pathogen. The type of pathogen involved provides important clues as to which component of the immune system may be defective and consequently guides the selection of relevant immunological tests (Table 36.2). While this targeted approach has much to commend it in defining immune phenotypes, integration with gene sequencing is increasingly used for definitive molecular diagnosis. Although this chapter is primarily devoted to PID, it is essential to consider and exclude the possibility of HIV infection as a driver for immunodeficiency in many of these clinical scenarios [17].

In view of the complexity of many immunological tests, it is essential that immunological investigations are performed under the guidance of a clinical immunologist to enable appropriate test selection, interpretation and advice on clinical management.

Management of Immunodeficiency

Infections in any immunodeficient patient should be treated aggressively with appropriate antimicrobial therapy. In patients with antibody deficiency, lifelong immunoglobulin replacement remains the cornerstone of management. For children with SCID, HSCT remains the main curative option, with the

Table 36.2 Patterns of infection as a guide to selection of immunological tests in suspected immune deficiency.

Type of pathogen	Consider	Relevant immunological tests
A – Encapsulated pathogens	Antibody deficiency Complement deficiency	Serum immunoglobulins, specific antibodies to polysaccharide and protein antigens Haemolytic complement activity
B – Viruses and intracellular pathogens	T-cell defect	Lymphocyte surface marker analysis Lymphocyte transformation
C – Combination of encapsulated pathogens and viruses and other intracellular pathogens	Combined B- + T-cell defect	As for A and B
D – Recurrent neisserial infection	Complement deficiency	Haemolytic complement activity
E – Recurrent staphylococcal abscesses and/or invasive fungal infections	Phagocyte defect	Neutrophil respiratory burst Leucocyte adhesion molecule expression (selected cases)

prospect of gene therapy for some forms of SCID. With the increasing use of genome sequencing for diagnostic characterisation of PID, there is an emerging role for allogeneic HSCT in carefully selected adult patients. As in children, HSCT can be curative, but careful patient selection and optimal timing of transplantation are critical for success [18]. Patients with complement deficiency should be fully immunised with the full range of available vaccines against neisserial, pneumococcal and *Haemophilus* infections. However, it is vital to avoid the use of live vaccines in any patient with immunodeficiency, in view of the real risks of vaccine-associated disease, as exemplified by vaccine-induced poliomyelitis in XLA and bacillus Calmette–Guérin (BCG)-induced mycobacterial disease in SCID.

Immunoglobulin Therapy

Therapeutic immunoglobulin is a blood component prepared from the plasma of 10 000–15 000 donors. The broad spectrum of antibody specificities contained in pooled plasma is an essential ingredient underpinning the success of intravenous (IvIg) and, more recently, subcutaneous immunoglobulin (SCIg) in infection prophylaxis in patients with antibody deficiency. Evidence from longitudinal studies in large cohorts of antibody-deficient patients and a meta-analysis of studies of IvIg replacement have highlighted the inverse correlation between trough IgG levels and the frequency of infection [19]. A similar inverse relationship between incidence of infections and steady-state IgG levels has recently been confirmed in studies of SCIg in patients with PID. In addition to its role in antibody replacement, the success of high-dose IvIg in the treatment of ITP has led to a veritable explosion in its use as a therapeutic immunomodulator in many autoimmune diseases spanning multiple specialties (Table 36.3).

The mechanisms of action of high-dose IvIg in autoimmune disease are complex and reflect the potent immunological actions of the different regions of an IgG molecule. It is helpful conceptually to consider the potential mechanisms of action in relation to the variable regions of IgG (F(ab′)2), the Fc region and the presence in IvIg of other potent immunomodulatory substances other than antibody (Figure 36.4). In ITP, the traditional view of Fc receptor blockade as the predominant mechanism by which IvIg is effective has recently been complemented by evidence from murine studies showing that IvIg-mediated amelioration of ITP is crucially dependent on interactions with the inhibitory FcγRIIB as well as the activating receptor, FcγRIII. Evidence from murine studies indicates that upregulation of FcγRIIB expression occurs via the small sialylated immunoglobulin component of polyclonal IvIg (estimated at 1–3%). This observation has led to the hypothesis that the use of small doses of concentrated sialylated immunoglobulin might be as efficacious as the use of conventional high-dose immunoglobulin for immunomodulation [20]. Subsequent *in vitro* and *in vivo* studies in mice and humans on the role of sialylation have proven contradictory, leaving this hypothesis unproven [21].

Immunoglobulin Replacement in Secondary Antibody Deficiency

IvIg replacement is beneficial in prophylaxis against infection in selected patients with secondary antibody deficiency associated with B-cell lymphoproliferative disease and myeloma. The predictors of response to IvIg are the presence of hypogammaglobulinaemia accompanied by low concentrations of pneumococcal antibodies and a failure to respond to test immunisation with pneumococcal polysaccharide (Pneumovax®). While IvIg is clinically efficacious in patients fulfilling these criteria, questions remain regarding its overall cost-effectiveness [22]. For this reason, IvIg replacement should be reserved for those patients who have failed a trial of prolonged antibiotic prophylaxis [23]. Despite evidence supporting the use of IvIg in secondary antibody deficiency, in practice its use

Table 36.3 Use of intravenous immunoglobulin (IvIg) as an immunomodulatory agent.

Disorder	Comments
Neurology	
Guillain–Barré syndrome	Treatment of choice and as efficacious as plasmapheresis (RCT, CR)
Multifocal motor neuropathy	Treatment of choice (RCT)
Chronic inflammatory demyelinating polyneuropathy	As an alternative to steroids (RCT)
Dermatomyositis	As an adjunct to immunosuppressive therapy (RCT)
Myasthenia gravis	For myasthenic crises (RCT)
Lambert–Eaton syndrome	For non-cancer-associated cases that have failed to respond to standard therapy (RCT)
Stiff-person syndrome	For severe cases unresponsive to standard therapy (RCT)
Autoimmune encephalitides	As an adjunct to steroids and plasmapheresis
Haematology	
Immune thrombocytopenic purpura	Selected cases unresponsive to standard treatment (RCT)
Covid vaccine-induced thrombosis and thrombocytopenia (VITT)	Treatment of choice alongside steroids and directly acting oral anticoagulants (case series)
Parvovirus-associated pure red cell aplasia	Selected cases
Paediatrics	
Kawasaki's disease	Treatment of choice (RCT)
Dermatology	
Autoimmune blistering disorders	Open studies/case series suggest benefit
Streptococcal/Staphylococcal toxic shock syndrome	Open studies/case series suggest benefit

Note: The list of indications is not exhaustive, but covers those disorders where IvIg is frequently used.
CR, evidence from Cochrane review; RCT, evidence from randomised controlled trials.

has not been widespread due to the advent of more immunogenic pneumococcal conjugate vaccines, coupled with improved overall management of these haematological malignancies. The indications for use of IvIg in secondary antibody deficiency have widened recently with its use as prophylaxis in patients with B-cell aplasia following chimeric antigen receptor therapy for B-cell malignancy [24].

Adverse Effects of Intravenous Immunoglobulin Therapy

Immediate Infusion-Related Adverse Effects

Minor to moderate immediate infusion-related adverse effects in the form of headaches, chills, rigors and backache occur in approximately 1% of patients irrespective of the therapeutic dose of immunoglobulin. These adverse effects are largely related to the rate of infusion and/or the presence of underlying infection in the recipient, and respond to a combination of a reduction in infusion rate coupled with simple analgesia. Very rarely, some patients with total IgA deficiency and pre-existing anti-IgA antibodies may develop anaphylaxis on exposure to IvIg preparations containing IgA. Although this risk may be minimised by the use of an IgA-depleted IvIg preparation in such patients, the exact role of anti-IgA is unclear, since many patients with high-titre antibodies may safely receive IvIg [25]. The demonstration of a novel splice variant of FcγRIIA in a patient

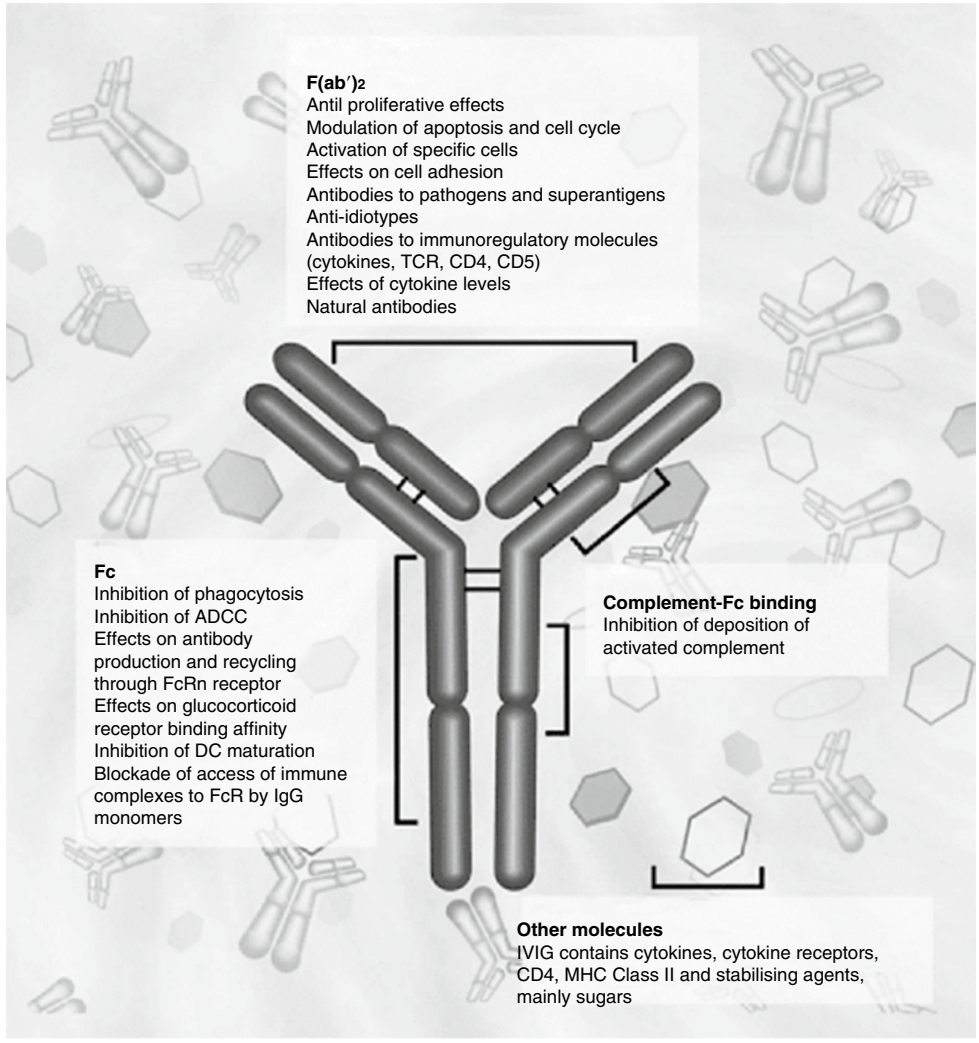

Figure 36.4 Immunomodulatory actions of intravenous immunoglobulin (IVIG). ADCC, antibody-dependent cell-mediated cytotoxicity; DC, dendritic cell; MHC, major histocompatibility complex. *Source:* Jolles S, Sewell WAC, Misbah SA. Clinical uses of intravenous immunoglobulin. Clin Exp Immunol 2005;**142**:1–11. Reproduced with permission of John Wiley & Sons.

with anti-IgA who experienced IvIg-induced anaphylaxis raises the possibility that activating receptors for IgG may be a contributory factor in triggering anaphylaxis [26].

Dose-Related Adverse Effects

The increasing use of IvIg for therapeutic immunomodulation has been associated with the development of a range of haematological, neurological, nephrological and dermatological adverse effects that are directly linked to the high doses (2 g/kg) required for autoimmune disease, in contrast to the low doses (0.4 g/kg) used for antibody replacement.

Haematological High-dose IvIg causes a dose-dependent increase in plasma viscosity [27], which is sufficient to precipitate serious arterial and venous thrombosis in patients with pre-existing thrombophilia, paraproteinaemia, severe polyclonal hypergammaglobulinaemia and atheromatous cardiovascular disease.

The risk of IvIg-associated acute haemolysis due to passive transmission of anti-blood group antibodies has been greatly minimised by the institution of rigorous quality control measures designed to ensure that the titre of anti-blood group antibodies in IvIg does not exceed 1 : 64. Despite these measures, there has been a recent resurgence in cases of IvIg-induced haemolysis, which appears to have coincided with the increasing use of more concentrated liquid preparations [28].

Neurological High-dose IvIg is associated with the development of self-limiting acute aseptic meningitis in a minority of patients (< 5%). Patients with background migraine are at higher risk, raising the possibility that meningeal irritation may be due to the interaction of exogenous IgG with meningeal endothelium.

Renal Nephrotoxicity due to high-dose IvIg is a particular risk associated with sucrose-containing preparations, which trigger osmotic tubular injury, leading to extensive vacuolar changes suggestive of historical cases of sucrose-induced nephropathy. The risk of renal damage is greatly minimised by avoiding the use of sucrose-containing IvIg preparations in patients with pre-existing diabetes and renal disease.

IvIg should also be avoided or used with caution in patients with mixed cryoglobulinaemia because of the real risk of the IgM component of cryoglobulin, containing rheumatoid factor reactivity, complexing with infused exogenous IgG to cause acute immune complex-mediated renal injury [29].

Dermatological A variety of cutaneous adverse effects including eczema, erythema multiforme, urticaria and cutaneous vasculitis may be triggered by high-dose IvIg. The relatively small number of cases reported to date does not enable any useful analysis that might help in minimising the development of dermatological adverse reactions.

Risks of Viral Transmission

Viral transmission is a risk with both low- and high-dose IvIg therapy. However, the increasingly stringent screening of donors coupled with the introduction of additional antiviral steps during plasma fractionation has greatly reduced but not eliminated the risk of HCV transmission with IvIg. For this reason, patients on maintenance IvIg should have their liver function monitored along with regular testing for HCV. The lack of any outbreaks of IvIg-associated HCV transmission since the last outbreak in 1993 [30] attests to the success of current viral safety measures. Unlike HCV, HIV and hepatitis B virus (HBV) have never been transmitted by IvIg, since the process of Cohn-ethanol fractionation specifically inactivates both of these viruses.

While recent reports of the development of variant Creutzfeldt–Jakob disease in recipients of blood from donors with asymptomatic disease have raised concerns of the possibility of prion transmission by blood components, this risk remains largely theoretical with IvIg. Leucocyte reduction and the use of plasma from countries free of bovine spongiform encephalopathy are measures designed to minimise this risk in the UK.

Practical Aspects of Immunoglobulin Therapy: Product Selection and Safe Use

The availability of several different preparations of IvIg (at least six in the UK at present) has raised the question of whether IvIg should be considered a generic product. For the purposes of antibody replacement, it is reasonable to consider the different products as equally efficacious, since each product is required to fulfil the stringent criteria laid down by the WHO for therapeutic immunoglobulin. With regard to the use of high-dose IvIg as an immunomodulator, studies comparing the efficacy of different products in Kawasaki's disease and chronic inflammatory demyelinating polyneuropathy (CIDP) have shown no difference in efficacy. Hence, while it would be reasonable to consider IvIg generic in terms of clinical outcomes [31], because of differences in the manufacturing

process and its impact on opsonic activity, Fc receptor function and complement fixation, it is best to regard individual products as distinct entities. In view of this and the potential difficulty in tracking any future outbreak of IvIg-associated viral transmission, it is prudent to maintain patients requiring long-term treatment on the same IvIg product, irrespective of whether IvIg is being used for antibody deficiency or immunomodulation.

Box 36.2 provides a useful checklist for the safe use of high-dose IvIg, including advice on product selection. Advice on individual products should be sought from a clinical immunologist.

Subcutaneous Immunoglobulin

Following comparative trials, the subcutaneous route of immunoglobulin delivery has been shown to be as efficacious as IvIg in infection prophylaxis in patients with primary antibody deficiency [32]. In practice, SCIg has proven to be popular with both patients and clinicians in view of its ease of use in patients with poor venous access and

Box 36.2 Checklist for the use of high-dose intravenous immunoglobulin (IvIg).

1. Prior to first infusion

Check renal and liver function, full blood count, viscosity, serum C-reactive protein, serum immunoglobulins and electrophoresis. Take blood for hepatitis C serology (not necessary to delay treatment while awaiting result) and save aliquot of frozen serum

Normal renal and liver function and serum IgA	Impaired renal function	Total IgA deficiency (< 0.05 g/L)	Partial IgA deficiency	IgM/IgG paraprotein	Patients at risk of hyperviscosity: > 4 cp (i.e. serum IgG > 50 g/L or with serum IgM > 30 g/L) or with background arterial disease
Proceed with any IvIg product	Avoid sucrose-containing IvIg and exercise caution; suggest using 0.4 g/kg/daily for 5 days and slower rate of infusion (suggest halving rate) Check creatinine daily before repeat dose is given	Use IvIg product containing low IgA content Check anti-IgA antibodies (time permitting, not essential)	Proceed with any IvIg product	Consider possibility of mixed cryoglobulinaemia Seek immunological advice before proceeding with IvIg	Exercise caution: use slower rate of infusion (suggest halving rate) and check viscosity at end of course

2. Adhere to the manufacturer's recommendations regarding reconstitution and rate of infusion

3. Record batch number of product

minimal adverse effects, in comparison with IvIg (Table 36.4). Using a weekly infusion regimen, SCIg achieves steady-state IgG levels without the peaks and troughs associated with IvIg. When patients transfer from IvIg to SCIg, the achievement of equivalent or higher steady-state levels with the same dose of SCIg reflects the reduced catabolism with subcutaneous delivery.

The success of SCIg as replacement therapy in antibody deficiency has led to its increasing use for immunomodulation, as in inflammatory neuropathy [33,34]. The use of multiple infusion sites in a motivated patient has enabled the delivery of higher doses required for immunomodulation. Using currently available 16% SCIg preparations, patients with autoimmune neuropathies are able to self-treat themselves with volumes of 200–220 mL weekly (32–35.2 g). The recent licensing of a 20% SCIg preparation and the future development of hyaluronidase-based preparations will enable the delivery of even higher doses and drive expansion of the immunomodulatory use of SCIg.

Table 36.4 Adverse effects of intravenous (IvIg) versus subcutaneous immunoglobulin (SCIg).

	SCIg	IvIg
Local reactions at site of infusion	Common (trivial)	Nil*
Anaphylaxis	–	Very rare*
Viral transmission (hepatitis C virus)	–	+†
Renal impairment	–	+
Aseptic meningitis	–	+
Thrombosis	–	+

* Possibly related to anti-IgA antibodies in some cases.
† Last outbreak in early 1990s.

KEY POINTS

1) Over 400 primary immunodeficiency disorders are currently recognised.
2) Common variable immunodeficiency is the most common acquired treatable immunodeficiency.
3) Intravenous (IvIg) or subcutaneous immunoglobulin (SCIg) is the mainstay of treatment for patients with antibody deficiency.
4) Haemopoietic stem cell transplantation remains the main curative option for children with severe combined immunodeficiency (SCID).
5) High-dose IvIg is widely used as a therapeutic immunomodulator in a range of autoimmune diseases.

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37 Development of a Patient Blood Management Programme

Jonathan H. Waters

Departments of Anesthesiology & Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

Patient blood management (PBM) is the timely application of evidence-based medical and surgical concepts designed to maintain haemoglobin concentration, optimise haemostasis and minimise blood loss in an effort to improve patient outcome [1]. The first bloodless medicine and surgery programmes were created in the 1980s to care for Jehovah's Witness patients. Over the next decade, it became apparent that outcomes for patients treated without allogeneic transfusion were equivalent to and sometimes better than those of patients cared for with allogeneic transfusion. In conjunction with the creation of these bloodless programmes, studies associating allogeneic transfusion with several adverse outcomes were published. Recognising that bloodless medicine and surgery might be good for all patients, the domain of PBM arose. In essence, a PBM programme recognises that allogeneic transfusion may be lifesaving but, like any medical therapy, is associated with risks. As such, it should be reserved for circumstances in which a clear benefit will result.

This chapter describes a structure for how a hospital might implement a PBM programme. Table 37.1 outlines a six-step strategy for implementing such a programme. The following discussion highlights this strategy.

Step 1: Leverage Computerised Physician Order Entry Systems to Guide Evidence-Based Transfusions

Over the last decade, multiple studies have suggested that more restrictive use of allogeneic blood results in no difference in patient outcome compared to patients transfused more liberally, but leads to avoidance of certain complications associated with transfusion and does so at a reduced cost of care [2]. These results have been demonstrated in a wide variety of patient populations, including critically ill intensive care, paediatric critical care and geriatric patients, and patients with orthopaedic hip fracture, gastrointestinal bleeding, cardiac surgery, septic shock and traumatic brain injury. Authors of a recent meta-analysis concluded that a restrictive strategy significantly reduced cardiac events, rebleeding, bacterial infections and mortality [3]. Also, these studies suggest that little benefit is gained from transfusing patients with haemoglobin levels above the 7–8 g/dL range.

Given that allogeneic transfusion is costly and provides no benefit to aggressive transfusion, a key component of a PBM programme is to drive clinicians to transfuse when evidence shows the possibility of patient benefit. Another big opportunity to

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Table 37.1 Strategy for developing a patient blood management programme.

Step 1	Leverage computerised physician order entry (CPOE) systems to guide evidence-based transfusions
Step 2	Reduce all forms of waste related to blood transfusion practices
Step 3	Promote alternative blood transfusion methods and systems
Step 4	Promote anaemia management strategies
Step 5	Limit iatrogenic blood loss
Step 6	Provide blood management education, awareness and auditing for clinicians

save money and improve outcomes is to reduce variability in transfusion behaviour. To do this, transfusion review committees have historically reviewed cases retrospectively. Most would consider this retrospective auditing limited in changing behaviour. More recently, the introduction of electronic medical records and computerised physician order entry (CPOE) systems has facilitated compliance with accepted standards. These systems allow for prospective monitoring of transfusion orders, as well as facilitating a process for monitoring transfusions that do not meet an institutional standard.

Figure 37.1 shows an order page from an electronic ordering system produced by Cerner (Kansas City, MO, USA) [4]. This page shows historical trends in the patient's haemoglobin levels and requires that a reason for the transfusion be chosen. If the transfusion order does not comply with institutional guidelines, an alert page is prompted, telling the clinician that they are deviating from the institutional standard. While the clinician can proceed with the transfusion, it leaves an auditing trail, which allows for the transfusion review committee to generate transfusion variance reports. Figure 37.2 shows such a report. On this report, service lines that are least compliant are identified so that focused education can be performed. Though not shown in Figure 37.2, it also allows for the opportunity to drill down to the specific clinician who is deviating from the institutional guideline.

Step 2: Reduce All Forms of Waste Related to Blood Transfusion Practices

The second step in starting a PBM programme should focus on waste reduction associated with transfusion practice. Waste throughout

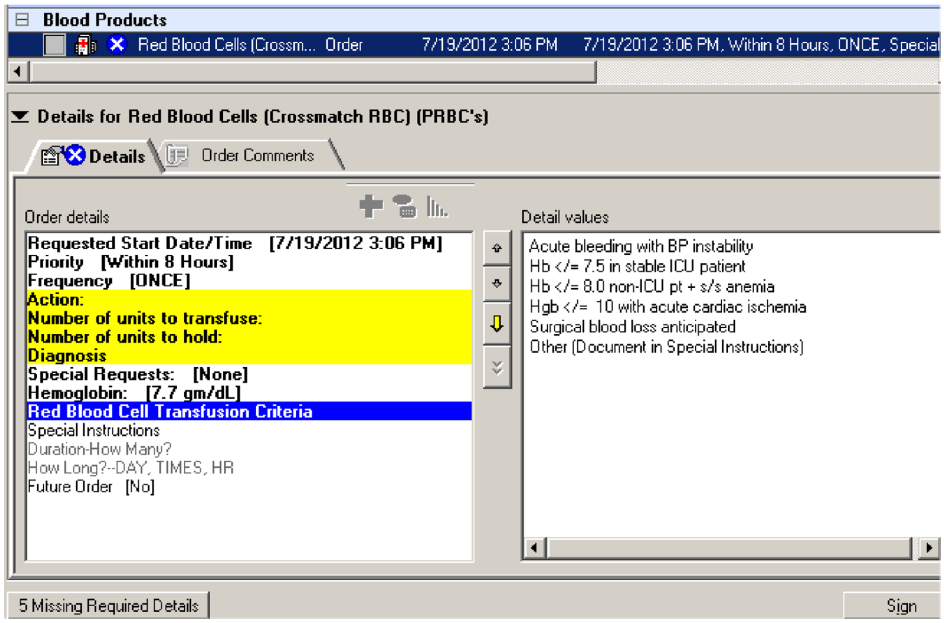
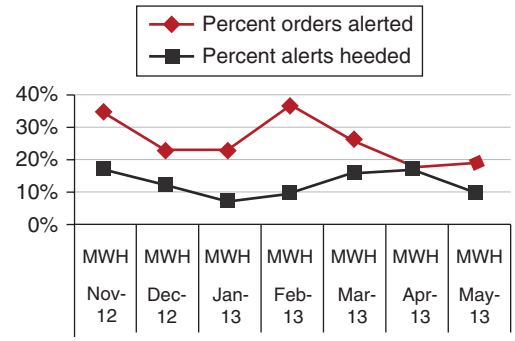


Figure 37.1 Computerised physician order entry page for red blood cells.

Facility	Total orders	Total alerts	Alerted orders not placed	Percent orders alerted	Percent alerts heeded
PUH	248	182	23	73.4%	12.6%
SHY	208	138	21	66.3%	15.2%
HAM	198	77	21	38.9%	27.3%
PAS	144	74	10	51.4%	13.5%
SMH	146	59	7	40.4%	11.9%
EAS	73	37	10	50.7%	27.0%
HZN	58	28	4	48.3%	14.3%
MER	275	27	4	9.8%	14.8%
NOR	39	23	0	59.0%	0.0%
MWH	103	20	2	19.4%	10.0%
MCK	62	12	3	19.4%	25.0%
BED	15	10	3	66.7%	30.0%

Total orders = Crossmatch Red blood cells, Red blood cells (Crossmatch RBC)
Percent orders alerted = Total alerts / Total orders
Percent alerts heeded = Alerted orders not placed / Total alerts



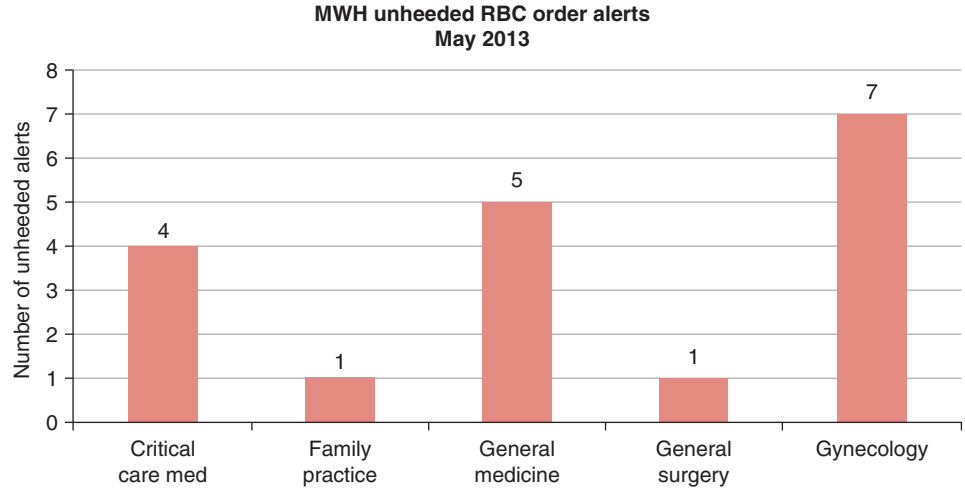


Figure 37.2 Transfusion variance report.

healthcare is rampant and provides a significant opportunity for cost savings [5]. Blood and blood products are wasted in many ways (Table 37.2). The practice of having a patient donate blood for themselves weeks prior to a surgical procedure, known as preoperative autologous donation (PAD), is one source of waste. Research using mathematical modelling and meta-analysis suggest that PAD increases instead of prevents transfusion; transfusion occurs at a higher rate than when no PAD has taken place [6,7]. In addition, at least 50% of these units are never used [8]. Thus, many major medical centres no longer coordinate PAD for surgical patients.

Crossmatching too many patients is another source of waste. Crossmatching is performed at a rate significantly greater than actual transfusions. Monitoring over-crossmatching is generally performed with the crossmatch to transfusion (C : T) ratio. A general standard is that the C : T ratio should be less than 2. Recently, the introduction of a computer crossmatching process for patients without antibodies allows crossmatched

Table 37.2 Sources of waste associated with blood transfusion.

Preoperative autologous donation (PAD)
Inappropriate transfusions
Excessive phlebotomy
Elevated crossmatch to transfusion ratio (C : T)
Wasted blood products
Dispensing of blood to the operating room

blood to be available for transfusion much more rapidly than if a serological crossmatch was necessary. An electronic crossmatch can be performed in less than five minutes, which means that a unit of blood can be made available at the patient bedside sooner and is not delayed because of the crossmatching process. Education as to the existence of the electronic crossmatch and its availability and speed can drive down the C : T ratio.

The Maximum Surgical Blood Ordering Schedule (MSBOS) is another cause of elevated C : T ratios. The MSBOS was developed so that a patient would not arrive in the operating room (OR) without adequate blood being available. The original version of the MSBOS was derived by the consensus of surgeons performing the procedure. In many institutions, the MSBOS has not been updated to reflect changes in surgical procedures. As such, an attempt to generate a data-driven MSBOS should be made [9]. While this might not be as relevant for centres with electronic crossmatching because blood is immediately available, it is useful in identifying patients for whom a presurgical type and screen process is necessary.

Excessive phlebotomy is another source of waste. An estimated 30% of transfusions that take place in an intensive care unit (ICU) compensate for excessive phlebotomy [10]. Over the course of a hospitalisation, a patient can be subjected to draws of hundreds of millilitres of blood. This starts with the 'rainbow draw' at admission. Figure 37.3 illustrates the concept of the rainbow draw, in which a well-intentioned nurse draws blood to fill vacutainer tubes of every colour, with the hope that a patient will not have to be re-phlebotomised when a fickle physician changes their mind about specific blood tests. In addition, the well-intentioned nurse may also draw double tubes of every colour just in case the laboratory rejects a specific sample. This excessive phlebotomy extends into the hospital stay, during which routine phlebotomy occurs every morning simply because the sun rises. As such, routine phlebotomy should not be allowed within a physician order entry system, so that the clinician is forced to order testing only when there is a clinical question to be answered.

Figure 37.3 The rainbow draw.

Blood mishandling also produces waste. This typically occurs when blood has been dispensed to a clinical area, but can warm or sit unused for a prolonged period. Blood waste can be significantly reduced by educating clinicians in appropriate handling practices. More importantly, the whole product ordering process needs to be re-engineered. Typically, blood transfusion laboratories distribute blood to a clinical area based on a physician order, but the nurse, who might be the one to administer the unit, may have multiple other patients to care for. In addition, the nurse may also need to administer premedication prior to the transfusion and ensure that a patent's intravenous line is available. One facility in which the blood bank distribution process was driven by a nurse demonstrated a 79% reduction in platelet wastage [11].

Lastly, waste arises from unnecessary ordering of blood to the OR. Having a cooler of blood at the patient bedside provides comfort to the anaesthesiologist and surgeon, but most of the blood delivered to the OR in this fashion goes unused [12]. Not only is this blood wasted, but also the blood transfusion laboratory needs to carry a larger inventory to keep these products sitting at the foot of an OR table.

Step 3: Promote Alternative Blood Transfusion Methods and Systems

In many surgical procedures such as multilevel spine fusion or open thoraco-abdominal aneurysm repair, heavy blood loss is highly probable. In strategising intraoperative management, reducing red cell transfusion through intraoperative blood recovery and reinfusion (cell salvage) should be considered, as well as normovolaemic haemodilution or component sequestration to reduce plasma and platelet transfusion.

Intraoperative blood salvage, more commonly called cell salvage, involves the collection of shed blood from the surgical field. The shed blood is then concentrated, washed, filtered and readministered to the patient. A rate of return of 60% of the lost cells can optimally be achieved with this technique. Using this technology, multiple complete blood volumes can be processed prior to needing allogeneic red blood cell supplementation [13].

Normovolaemic haemodilution entails the withdrawal of autologous blood prior to the start of the surgical procedure and replacing volume with asanguineous intravenous fluids [14]. The primary goal of this technique is to create a relative anaemia in the patient so that blood shed during the operative procedure effectively contains a reduced number of red cells. Once the threat of blood loss is diminished, the harvested cells are returned to the patient. A significant disadvantage to normovolaemic haemodilution is that it does not work very well to prevent red cell transfusion. The savings attributable to normovolaemic haemodilution are estimated at 100–200 mL, hardly enough to significantly reduce allogeneic exposure [15]. The value of haemodilution relates to its ability to treat coagulopathy that might develop during a major blood loss procedure. Intraoperative blood salvage allows for allogeneic red blood cell avoidance up to two to three blood volumes, while sequestered plasma and platelets from normovolaemic haemodilution protect the patient when a dilutional or consumption coagulopathy occurs. In general, removal of a litre of whole blood through normovolaemic haemodilution, and then reinfusion, is adequate for platelet and plasma transfusion avoidance [16].

Step 4: Promote Anaemia Management Strategies

Attention should be paid to the future surgical patient's haemoglobin concentration in the preoperative period. The prevalence of preoperative anaemia is striking. Preoperative anaemia has been documented to range from 5% in female geriatric hip fracture patients to over 75% in colon cancer patients [17]. Other studies have shown that anaemia exists in 34% of non-cardiac surgery patients and 35% of those undergoing total knee or hip replacement [18]. Preoperative anaemia is the greatest risk factor for perioperative transfusion. In addition, preoperative anaemia has been associated with higher mortality rates in surgical patients.

Optimisation of a patient's haemoglobin can take place using iron, vitamins and occasionally using erythropoietin (Figure 37.4). In general, determining the reason for the patient's anaemia is the best strategy. For instance, many patients undergoing joint replacement surgery are at an age where they are at risk for colon cancer. A patient with an occult colon cancer would be far better served by having the mechanism for their anaemia evaluated prior to a joint replacement, because they might more greatly benefit from a colon resection.

How anaemia management is structured should be tailored to the hospital or health system. Many hospitals use a nurse manager to individually manage patients, while larger hospitals might find this strategy cost prohibitive.

Step 5: Limit Iatrogenic Blood Loss

Limiting blood loss from phlebotomy is achieved through point-of-care testing devices. A wide variety of laboratory tests are available through point-of-care devices. These include blood gas, electrolyte, glucose level, haemoglobin concentration and coagulation function tests. Testing devices measuring these factors require microlitres of blood rather than the standard 3–4 mL necessary with standardised laboratory testing. Point-of-care testing allows for the treating clinician to make decisions based on quantitative data at the point of care. This is most valuable in the OR or ICU, where

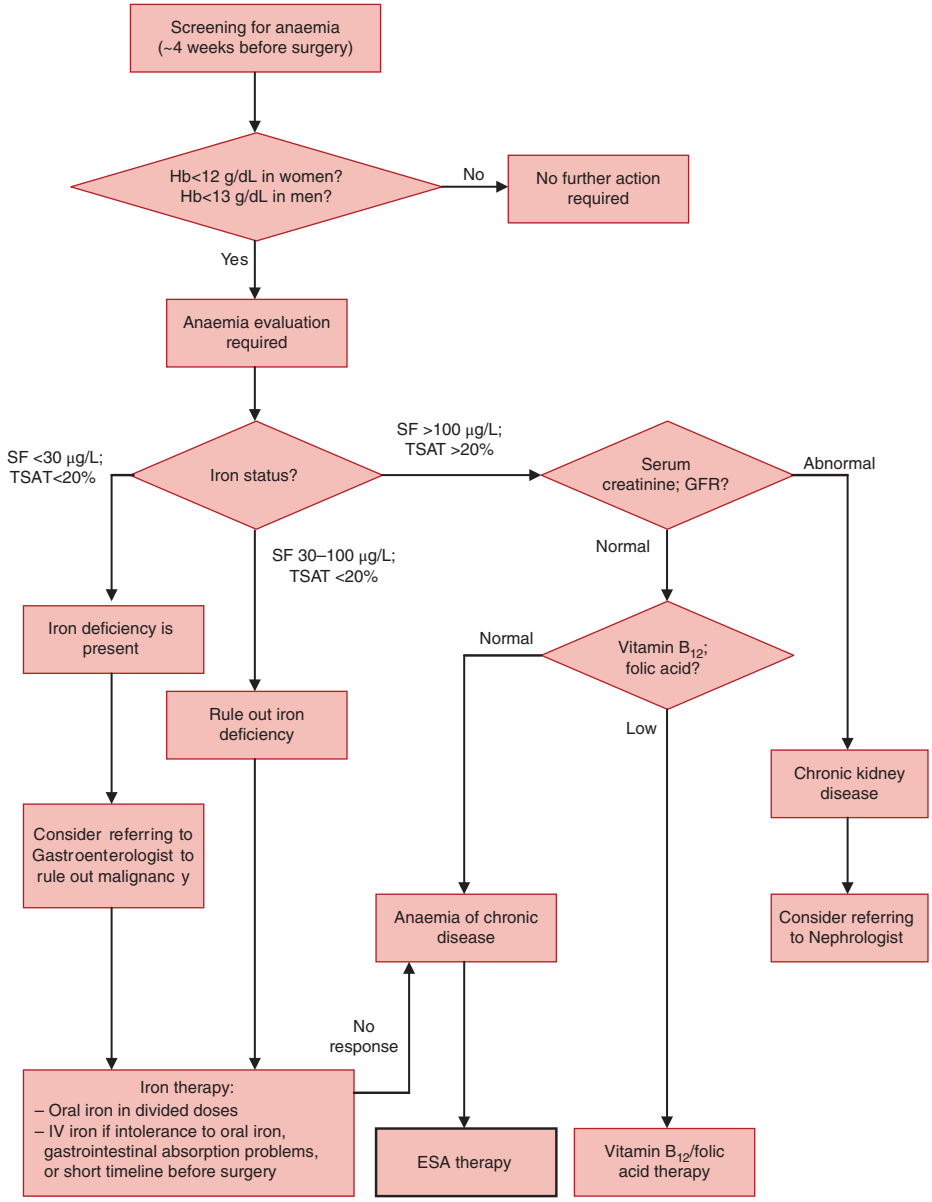


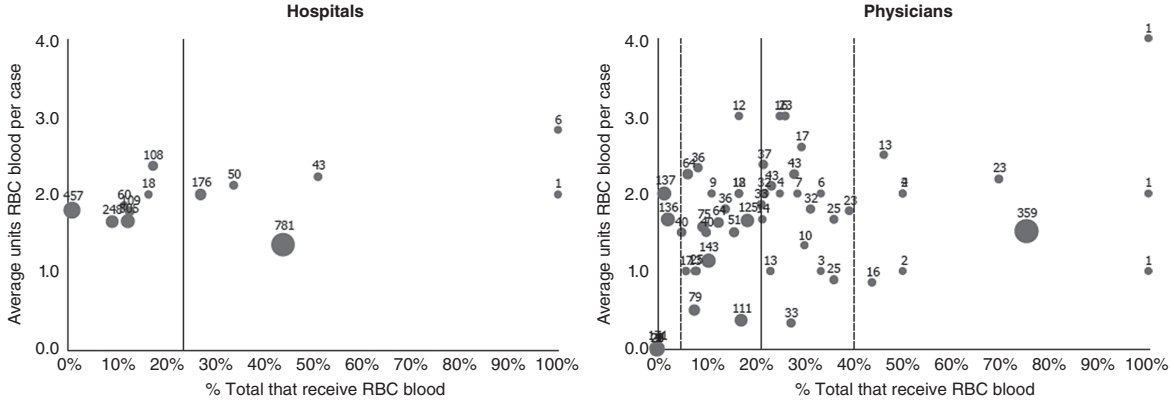
Figure 37.4 Diagnostic algorithm for determining the cause and treatment of an anemia.

transfusion needs are traditionally guessed. In cardiac surgery, where point-of-care testing has been implemented blood use has been reduced by up to 70% [19].

In addition to point-of-care testing devices, hospitals can use paediatric vacutainer tubes for routine laboratory testing. These tubes aspirate 0.5–1 mL of blood, which further reduces routine phlebotomy losses. While resistance to this practice is frequently encountered from laboratory staff, the focus should be on patient safety and not the convenience of the laboratory.

Another system that can reduce phlebotomy loss exists in conjunction with invasive lines. Routinely, 10 mL of blood is drawn through these lines prior to drawing the laboratory sample. The first 10 mL is then discarded. Systems have been implemented in which this drawn blood is sterilely aspirated and then reinjected following sampling.

(a)



	BED	EAS	HAM	HRY	HRZ	MAG	MCK	MER	NWH	PAS	PUH	SHY	SMH
Total Recv RBC blood	1	19	48	17	22	5	3	14	7	23	6	344	38
Surgical discharges	1	108	176	50	43	457	18	109	60	248	6	781	305
% Total Recv RBC blood	100.0%	17.6%	27.3%	34.0%	51.2%	1.1%	16.7%	12.8%	11.7%	9.3%	100.0%	44.0%	12.5%

	2013/Apr	2013/May	2013/Jun	2013/Jul	2013/Aug	2013/Sep	2013/Oct	2013/Nov	2013/Dec	2014/Jan	2014/Feb	2014/Mar
Surgical discharges	192	182	206	155	197	189	222	224	179	208	193	215
Total receiving RBC blood	58	48	51	39	34	52	53	50	34	39	48	41
% Total that receive RBC blood	30.2%	26.4%	24.8%	25.2%	17.3%	27.5%	23.9%	22.3%	19.0%	18.8%	24.9%	19.1%
Avg units RBC blood per transfused case	1.83	1.77	1.59	1.41	1.91	1.27	1.79	1.66	1.50	1.33	1.31	1.54
Total receiving TXA	103	98	106	79	95	100	126	130	112	132	137	143
% Total that receive TXA	53.6%	53.8%	51.5%	51.0%	48.2%	52.9%	56.8%	58.0%	62.6%	63.5%	71.0%	66.5%
Avg units TXA per transfused case	1.07	1.07	1.07	1.08	1.17	1.07	1.08	1.08	1.14	1.21	1.26	1.24
ALOS w blood	3.4	3.3	3.1	3.2	3.6	2.8	3.0	2.6	2.5	2.3	2.9	3.0
ALOS w/o blood	2.9	2.8	3.0	2.8	2.9	3.0	2.8	2.7	2.7	3.0	2.7	2.8
% Autologous	0.0%	0.0%	3.9%	2.6%	2.9%	0.0%	1.9%	0.0%	0.0%	0.0%	0.0%	0.0%

Figure 37.5 Benchmarking report.

(*Continued*)

(b)

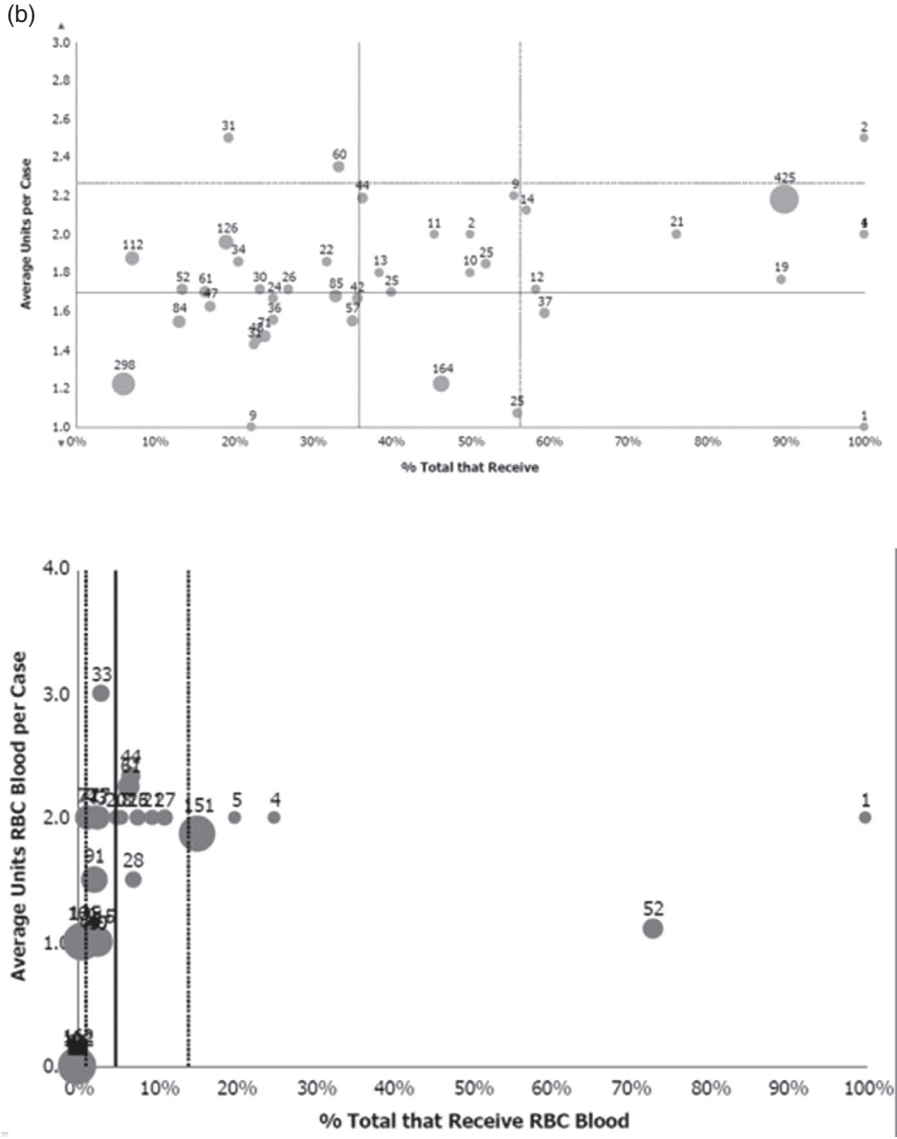


Figure 37.5 (Continued)

Step 6: Provide Blood Management Education, Awareness and Auditing for Clinicians

The last step in this PBM implementation strategy is to benchmark blood use for commonly performed surgical procedures. Figure 37.5 shows a report on blood use for primary total hip replacement procedures. There are two graphs. On both, the X-axis represents blood use by surgeon, with the Y-axis showing blood use per transfusion episode. Each bullet represents an individual surgeon, with the size of the bullet representative of the number of cases done during a reporting period. The top graph shows the variance when reporting first started, whereas the second graph shows five years later. This variability relates to multiple factors, including surgeon differences in tolerability of anaemia, surgical approach, deep venous thrombosis prevention practices and use of antifibrinolytics. By developing such reports and publicly sharing them, surgeons will self-regulate their transfusion behaviour.

Cost of Blood

While the risks of allogeneic transfusion are controversial, the cost of providing blood for patients is significant. When developing a PBM programme, it is important to understand the costs associated with transfusion to convince hospital administrators to support such a programme. The median acquisition cost for a leuco-reduced red cell unit was $207 (£148) in 2017 [20]. The total cost per red cell unit for an individual patient has been estimated to range from $726 to $1183 (£484–789), which includes all associated costs with getting a unit of blood to a patient bedside and transfusing it [21]. Most hospital administrators recognise that the blood bank will not disappear because of reduced blood use, so any cost savings calculations should relate to the acquisition cost and not the total cost.

KEY POINTS

1) The easiest method to reduce exposure to donor blood is to use a restrictive transfusion trigger for all blood products.
2) Consider red cell transfusion only if the haemoglobin concentration is 80 g/L or less in haemodynamically stable patients, including asymptomatic patients with stable cardiovascular disease.
3) Measures for patient blood management, e.g. for treating anaemia, minimising iatrogenic blood loss and intraoperative blood salvage, should be considered as alternatives to transfusion with donor blood.
4) Safe and appropriate blood use is facilitated using information technology throughout the transfusion process.
5) Computerised physician order entry (CPOE) systems can be leveraged to guide evidence-based transfusions.
6) All forms of waste related to blood transfusion practices should be addressed.
7) Education in patient blood management should be provided for both clinicians and patients.

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38 Perioperative Patient Blood Management

Martin Shao Foong Chong[1], *Martin Rooms*[1], *Ravishankar Rao Baikady*[1] *and Toby Richards*[2]

[1] *The Royal Marsden NHS Foundation Trust, London, UK*
[2] *University of Western Australia, Perth, Australia*

Preoperative anaemia affects 30–60% of patients presenting for major surgery and is an independent risk factor associated with increased blood transfusions, postoperative complications, delayed discharge and increased mortality [1,2]. Surgical bleeding contributes to the risk of anaemia and the need for allogeneic blood transfusion (ABT) [3]. 'Patient blood management' (PBM) was coined in 2008 as a pragmatic guide to avoid unnecessary blood transfusions and shift towards a more patient-focused approach in transfusion medicine [4].

PBM is defined as 'the timely application of evidence-based medical and surgical concepts designed to maintain hemoglobin concentration, optimize hemostasis and minimize blood loss in an effort to improve patient outcome' [5]. PBM management is built on the 'three pillars': optimise erythropoiesis; minimise blood loss (surgical) and bleeding (coagulopathic); and harness and optimise physiological reserve of anaemia. The three pillars of PBM align well to a patient's journey through surgery from preoperative preparation to intraoperative techniques and postoperative restrictive practice (Figure 38.1).

PBM was endorsed by the World Health Organization (WHO) and in the UK promoted by National Health Service Blood and Transplant (NHSBT) as part of a quality improvement programme in blood transfusion [6]. Over the last decade, the UK has seen a considerable reduction in the use of blood transfusion in surgery [7], mirrored in part by the increased development of laparoscopic/robotic surgery and minimally invasive interventions. However, it is in the setting of elective surgery, where anaemia, bleeding and transfusion are common, that increased knowledge, awareness and directed use of blood transfusion practice with PBM has been associated with reduced blood transfusion and improved patient outcomes [8].

In this chapter we review how the three pillars of PBM can be implemented within the preoperative, intraoperative and postoperative periods.

Anaemia and Surgery

The WHO defines anaemia as an insufficient circulating red cell mass with a haemoglobin concentration < 130 g/dL in males and < 120 g/dL in females. The prevalence of preoperative anaemia affects about a third of all patients undergoing non-day-case surgery, rising to 40–60% of those undergoing major cardiac and non-cardiac operations [9].

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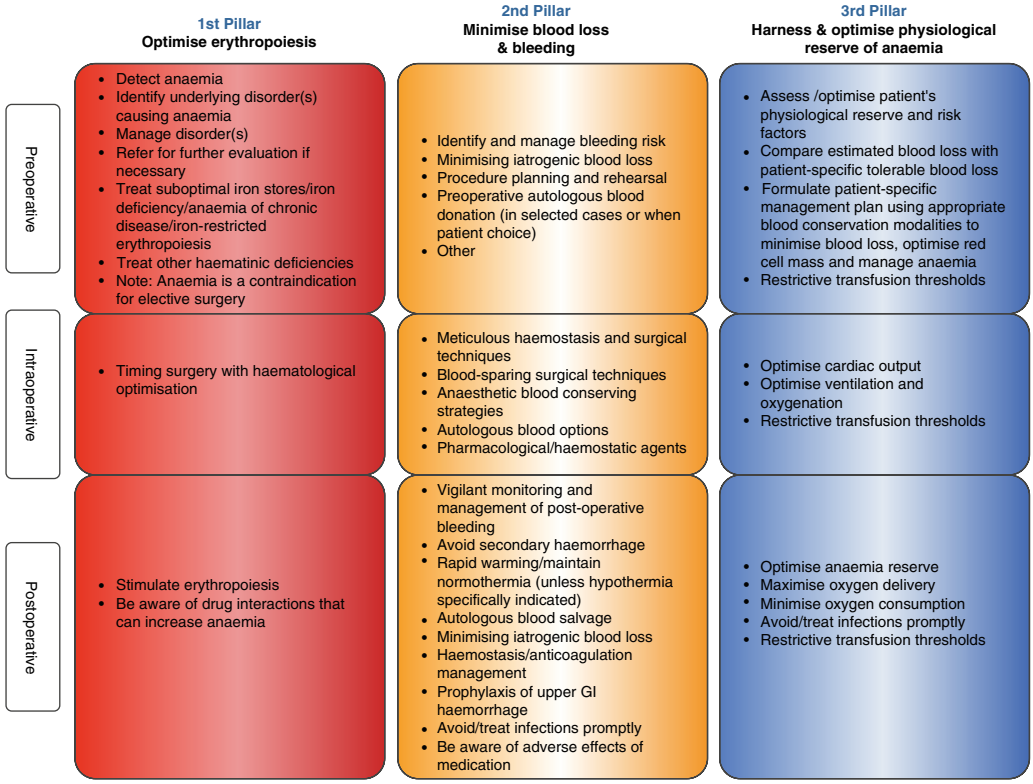

Figure 38.1 The three-pillar, nine-field matrix of perioperative patient blood management.

The most common cause of anaemia is iron deficiency, due to either nutritional deficiency or chronic blood loss. This leads to a state of absolute iron deficiency (AID), manifest as a decrease in total body iron stores, as reflected in low serum levels of the iron storage protein, ferritin. Also relevant to surgical patients is functional iron deficiency (FID), where inflammation, infection or malignancy can lead to iron sequestration despite the presence of adequate iron stores and, if prolonged, subsequently iron-restricted erythropoiesis, causing anaemia of chronic disease. The principal cause, inflammation, upregulates hepcidin, the master regulator of iron homeostasis, which reduces dietary iron absorption and limits iron transport out of cells, with retention of iron within macrophages, and consequently reduces circulating levels of iron and its availability for erythropoiesis [10]. Therefore, the treatment of iron-deficiency anaemia in surgical patients with oral iron is often ineffective and intravenous iron, which bypasses these hepcidin-mediated pathways, is a potential therapy.

Anaemia is an independent and potentially modifiable target for intervention to reduce perioperative morbidity and mortality. Even mild anaemia is associated with increased risk in the perioperative period of 30–40%, with a further relationship of more severe anaemia with worse outcome [1]. The EuSOS survey demonstrated that moderate or severe anaemia was associated with increased in-hospital mortality (odds ratio [OR] 1.99 and 2.28, respectively) [11]. However, preoperative anaemia is often identified late in the perioperative pathway [7]. International treatment guidelines recommend that patients undergoing surgery with an expected blood loss of

> 500 mL should be screened for anaemia at least two weeks before surgery, and anaemia should be identified and managed [2,12].

Blood Transfusion

Transfusion of packed red cells has historically been the solution for anaemia in the perioperative period. However, replacing red cells does not address the underlying cause of anaemia, so in this aspect blood transfusion should not be regarded as a treatment [9].

Red cell transfusion is costly. In the UK, a single unit of red cells costs £190. Aside from the fact that blood is a precious resource, it is also not without risk. While the risk of transfusion reaction and transmissible infection is small but recognised, other risks such as febrile reaction and transfusion-related circulatory overload (TACO) are relatively common and should not be overlooked [13].

Blood transfusion is an independent risk factor for perioperative morbidity and mortality in surgical patients. Various studies have found a dose-dependent relationship between ABT and stroke, pulmonary, renal, septic, thromboembolic and wound complications [14]. Moreover, concerns have been raised about transfusion-induced immunomodulation and its possible role in postoperative bacterial infection and cancer recurrence [15]. As with all interventions, the timely and appropriate use of blood transfusion is important for quality of care, and complications may be more common in those patients where transfusion may not be the appropriate therapeutic choice.

Patient Blood Management in Surgical Practice

Preoperative Optimisation

Preoperative assessment is key in risk stratification, to identify patients with anaemia, risk of bleeding and likelihood of needing blood transfusion. The European Society of Anaesthesiology guidelines recommend that haemoglobin be measured ideally 3–8 weeks before major elective surgery in patients with an increased risk of blood loss to allow for adequate time for treatment of anaemia, if necessary, before surgery [16]. Identification of the high-risk patients at preassessment clinic should trigger a directed review of the patient to manage preoperative, intraoperative and postoperative PBM strategies.

Increasing numbers of patients take anticoagulant and antiplatelet agents, and an individualised assessment of the thrombotic risk of stopping these agents must be balanced against the risk of perioperative bleeding. Bridging therapy may be required to substitute long-acting anticoagulants (e.g. warfarin) with shorter-acting anticoagulants (e.g. heparin), but often only in high-risk patients. Patients on direct oral anticoagulants or dual antiplatelet therapy can discontinue these in a timely manner preoperatively to allow new clotting factors or platelets to be generated [17]. Despite national guidelines, in the NHSBT audit nearly a third of patients on warfarin underwent an operation with an international normalised ratio (INR) > 1.4, due to erroneous timing for omissions of warfarin and issues with low molecular weight heparin bridging regimes [7]. Reversal strategies should be in place, such as the use of vitamin K intravenously 24 hours before surgery for patients on warfarin or directed antidote therapy for patients undergoing emergency surgery. Consultation with Haematology, Pharmacy and/or Transfusion Medicine should ensure protocols are in place for reversal strategies in anticoagulated patients requiring urgent intervention. A key feature for the PBM plan is to ensure timely restarting of anticoagulation or antiplatelet therapy in the postoperative period.

In patients undergoing major surgery where moderate to high blood loss, defined as more than 500 mL, is likely, or > 10% probability for blood transfusion [16], anaemia should be screened for and investigated.

The presence of haemoglobin (Hb) < 130g/L should automatically trigger laboratory testing for serum ferritin, transferrin saturation (TSAT), C-reactive protein (CRP, a marker of inflammation) and renal function [18], as well as vitamin B_{12} and folate levels [12].

Iron-deficiency anaemia can be diagnosed with iron studies; bone marrow iron assessment is for the most part obsolete. Serum ferritin < 30 ng/mL is the mainstay for diagnosing AID and has a sensitivity of 93% and specificity of 75% [10]. TSAT < 20% is useful to define low plasma iron availability to tissues in both AID and FID. FID is defined by low circulating iron levels and TSAT < 20%, but with normal or increased ferritin levels > 100 ng/mL, along with systemic markers of inflammation, e.g. CRP (Figure 38.2).

Oral iron supplementation is low cost and can replenish iron stores prior to surgery. Oral iron formulations are predominantly ferrous salts (e.g. ferrous sulphate or gluconate), but compliance can be a problem due to gastrointestinal symptoms, particularly constipation, nausea and diarrhoea. Oral iron has relatively poor absorption of only 10–15% or 150–200mg per month in total and will take at least 8–12 weeks to show effectiveness. Consideration should be given to using oral iron as the first-line treatment for iron-deficiency anaemia in patients undergoing elective non-urgent surgery where timelines are appropriate, such as orthopaedics. However, in some surgical patients, FID may the cause of anaemia and the timelines for surgery are often only a couple of weeks, particularly for cancer cases, so the role for oral iron therapy is limited.

The use of intravenous iron therapy has increased significantly in the last decade. Parenteral iron preparations bypass the effects of hepcidin on gastrointestinal absorption and macrophage sequestration. Safety of parenteral iron has a historical concern based on adverse events from obsolete high molecular weight iron dextran formulations. With changes in the preparations, serious hypersensitivity reactions (anaphylaxis) with modern parenteral iron are now rare, occurring at a rate of 10–20 per 100 000. The more modern therapies enable a full treatment dose of 1000 mg or more, such that

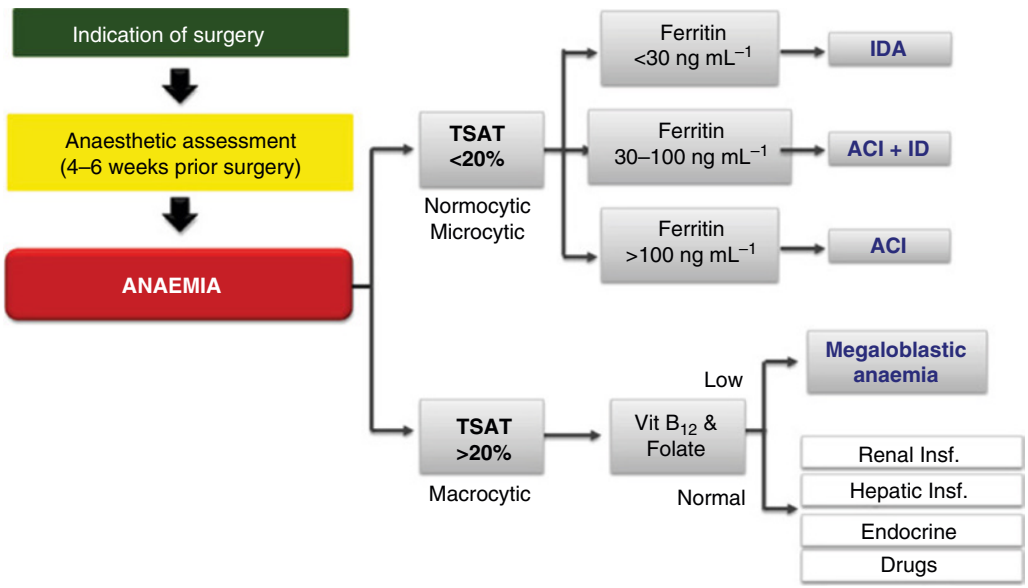

Figure 38.2 Flowchart for anaemia classification. ACI, anaemia of chronic inflammation; ID, iron deficiency; IDA, iron deficiency anaemia; TSAT, transferrin saturation. *Source:* Munoz et al. 2015 [18]. Reproduced with permission of Oxford University Press.

Table 38.1 Intravenous iron formulations for total single-dose infusion.

Formulation	Ferric carboxymaltose	Iron isomaltoside
Brand name	Ferinject®/Injectafer®	Monofer®/Monoferro®
Iron content (mg/mL)	50	100
Maximum single dose	20 mg/kg (max 1000 mg)	20 mg/kg (max 1600 mg)
Dosage	Single-dose infusion	Single-dose infusion
Duration of infusion	15 minutes	15 minutes (< 1000 mg) 35 minutes (> 1000 mg)

complete restoration of iron reserves can be achieved in a single 15–30-minute infusion (Table 38.1). Intravenous iron presents a safe, efficacious and effective treatment for patients with iron deficiency.

Intravenous iron therapy has become a recognised therapy as part of PBM in surgery. It seemed plausible and logical that treatment of surgical patients would help improve haemoglobin concentrations, reduce the associated morbidity and mortality of preoperative anaemia [19] and reduce the requirement for allogeneic blood transfusions. To test this hypothesis, the Preoperative intravenous iron to treat anaemia before major abdominal surgery (PREVENTT) trial was performed. This randomised, double-blind controlled trial compared preoperative intravenous iron to placebo in patients 10–42 days before major abdominal surgery. The primary outcome was the need for blood transfusion (and death), with secondary outcomes of patient complications, length of hospital stay and recovery [20]. The trail was performed in the UK in line with NHS timelines for surgery, with patients receiving intervention a median 15 days (range 12–22) before surgery and transfusion practice in line with NHSBT protocols. The primary results showed no difference in outcome for blood transfusion or perioperative outcomes for patients in either the intention to treat (ITT) and per protocol (PP) or predefined subgroup analyses. The results were a surprise, but in the COVID-19 pandemic where preoperative assessment was limited, it was reassuring that patients were not being disadvantaged by not having preoperative intravenous iron.

An important finding from PREVENTT was that patients in the intravenous iron therapy arm had significantly higher haemoglobin concentrations (mean difference 10.7 g/L, 95% confidence interval [CI] 7.8–13.7) after surgery at eight weeks, and this was associated with a significant reduction in hospital readmissions for surgical complications. This confirms the efficacy of the intervention and raises questions about the role and timing of intravenous iron in the surgical PBM pathway of care that warrant further investigation [21].

However, the routine practice of giving generic intravenous iron preoperatively to patients with anaemia (or FID) does not appear to be effective [21,22]. Nevertheless, an individualised role should be adopted to ensure the appropriate management of AID in patients where oral iron does not work or is not tolerated. The result of PREVENTT also highlights the need to better understand FID and the role of therapy.

In the last decade, the role of recombinant human erythropoietin (rEPO) has become reduced outside of renal medicine. It has more recently been explored as an adjunct for PBM. Meta-analyses of rEPO in patients undergoing cardiac or orthopaedic surgery suggest that rEPO can increase Hb levels and reduce the number of patients requiring ABT [23,24]. Guidelines from the Network for Advancement of Transfusion Alternatives

(NATA) recommend that rEPO should be used for anaemic patients in whom nutritional deficiencies have been corrected or excluded in orthopaedics [25]. The benefits of ultra-short-term combination treatment with ferric carboxymaltose, erythropoietin alphas, vitamin B_{12} and folic acid have been shown to increase Hb concentration and reduce red blood cell (RBC) transfusion in patients undergoing urgent cardiac surgery [26]. However, as with PREVENTT, no change in patient outcomes was seen.

These results of a one-size-fits-all approach to treat preoperative anaemia highlight the need for patients to be investigated and managed in a directed and individualised manner before surgery. Further work is needed on the mechanisms of FID and the role of combination treatment or novel therapies that interact with hepcidin or erythropoietin production.

Intraoperative Management

Intraoperative management of blood loss may be influenced by surgical technique, anaesthetic strategies and pharmacological management. Transfusion, when required, should be guided by point-of-care viscoelastic haemostatic assay testing and/or conventional laboratory assessments. Surgical technique is the key factor in determining perioperative blood loss. Use of minimally invasive techniques such as laparoscopic, robot-assisted and endovascular procedures helps in minimising tissue trauma, reduces blood loss and decreases transfusions synergistic to the goal of PBM. Advances in technology have also ensured that meticulous haemostasis can be achieved by use of electrical diathermy, laser cautery as well as adjunct topical haemostatic agents (fibrin sealants that contain fibrinogen and thrombin or topical tranexamic acid [TXA]), or local anaesthetic with adrenaline as a vasoconstrictor.

Cell salvage is advocated where the anticipated blood loss is > 1000 mL and is an established technique in cardiac, orthopaedic, obstetric and major vascular surgery [27]. In cell salvage, blood is collected via a double lumen suction device, stored within a reservoir with added anticoagulants, then washed, filtered, suspended in saline and returned to the patient. Advances in equipment have shown cost effectiveness with as little as 200 mL of blood returned. In a recent meta-analysis, intraoperative cell salvage has been associated with a reduction in ABT, infection rate, length of hospital and mortality [28]. Although there have been concerns around reintroduction of unwanted bacteria or malignant cells in salvaged blood, studies have shown reinfusion of autologous salvaged blood to be comparable to allogeneic blood in terms of safety and not found to promote postoperative sepsis, tumour dissemination or distant metastasis [29].

Acute normovolaemic haemodilution (ANH) is another autotransfusion technique where whole blood is drained by gravity into blood collection bags before surgery, with concurrent infusion of crystalloid fluids to maintain normovolaemia, then the collected blood is retransfused at the end of surgery. It is particularly useful in cardiac surgical patients in reducing ABT and has been shown in meta-analysis to be cost-effective [30]. ANH is accepted by most Jehovah's Witnesses. However, it is not a common practice in most countries due to various logistical issues and wastage of collected blood.

The maintenance of a balanced physiology by anaesthetists throughout the intraoperative period is integral to avoiding the lethal triad of hypothermia, coagulopathy and acidosis. Hypothermia impairs fibrinolysis synthesis. Strict temperature control using fluid warmers, forced air blankets and heated mattresses should be employed routinely to avoid a core body temperature below 35 °C. When appropriate, blood gases should be assessed so that any acidosis (pH < 7.2) and hypocalcaemia (ionised calcium < 1 mmol/L) can be identified and treated. Manipulation of cardiovascular physiology to allow a clear surgical field for faster completion of a procedure may be required through permissive

hypotension in trauma patients or lowering central venous pressure in hepatic resection. Careful patient positioning with the patient slightly head up will help with venous drainage and improve surgical operating conditions. Personalised haemodynamic management strategies, using goal-directed fluid therapy and cardiac output monitors, help to optimise cardiac output and therefore oxygen delivery.

Transfusion management of major haemorrhage is essential in controlling coagulopathy and haemostasis. Successful management of major haemorrhage requires a protocol-driven multidisciplinary team approach with a practical algorithm for transfusion management [31]. The design of this plan should weave in appropriate PBM techniques to maximise the efficacy of efforts to conserve blood and manage bleeding, as well as provide information on the use of blood products to replace lost blood volume (Figure 38.3).

The underlying mechanisms of acute traumatic coagulopathy are highly complex and dynamic. Major traumatic haemorrhage initiates dysregulation and hyperactivation of the activated protein C pathway, which promotes anticoagulation and hyperfibrinolysis. Transfusion of large volumes of RBCs and other intravenous fluids that contain no coagulation factors or platelets causes 'dilutional coagulopathy'. In the setting of haemorrhage, plasma fibrinogen falls to subhaemostatic levels (< 1.5 g/L) after 1–1.5 blood volume replacement. The platelet count usually remains above 50×10^9/L until 1.5–2.5 blood volumes have been replaced [31].

When undertaking transfusion in a patient suffering a major haemorrhage, the concept of a balanced ratio of RBC, plasma and platelets in a 1 : 1 : 1 ratio in the initial resuscitation has been extrapolated from military to civilian practice in the setting of traumatic injury. The benefits of early transfusion of fresh frozen plasma (FFP) or plasma in a fixed ratio to RBCs ('shock packs') in traumatic haemorrhage was proposed following the observational PROMMTT trial [32] and randomised controlled PROPRR trial [33], to reverse coagulopathy and reduce bleeding. However, large-volume plasma transfusion carries increased risks of circulatory overload (TACO), allergic reactions and transfusion-related acute lung injury (TRALI), and further research is needed to clarify its role, particularly in non-trauma bleeding populations.

Once laboratory values are available, the recommendation is to switch to goal-directed resuscitation for bleeding patients. When fibrinogen levels fall below 1.5 g/L, cryoprecipitate or fibrinogen concentrates can be used for fibrinogen replacement. When comparing the two products, there is no good evidence for superiority, but both provide a more concentrated source of fibrinogen than plasma transfusion. Cryoprecipitate has additional clotting factors, including von Willebrand factor, while fibrinogen concentrate is quicker to administer but more expensive. The use of other therapies, such as prothrombin complex concentrates or recombinant factor VIIa, may be considered for selected indications [34].

Pharmacological treatments with early administration of antifibrinolytics, specifically TXA, help by preventing clot breakdown without inducing clotting and have been shown by the CRASH-2 trial to improve the survival of patients with major traumatic haemorrhage [35]. TXA has a good safety profile, is readily available and inexpensive. TXA given prophylactically as 1 g at induction has demonstrated benefit in reducing blood loss and ABT in surgery associated with significant blood loss such as obstetric and cardiac surgery [36,37].

Transfusion, when employed, should be guided by rapid conventional assays or point-of-care testing. Viscoelastic haemostatic assays (VHAs), i.e. thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®), measure changes in clot tensile strength over time, providing information on the dynamics of clot formation, clot strength and lysis. This enables rapid diagnosis of specific abnormalities in coagulation to

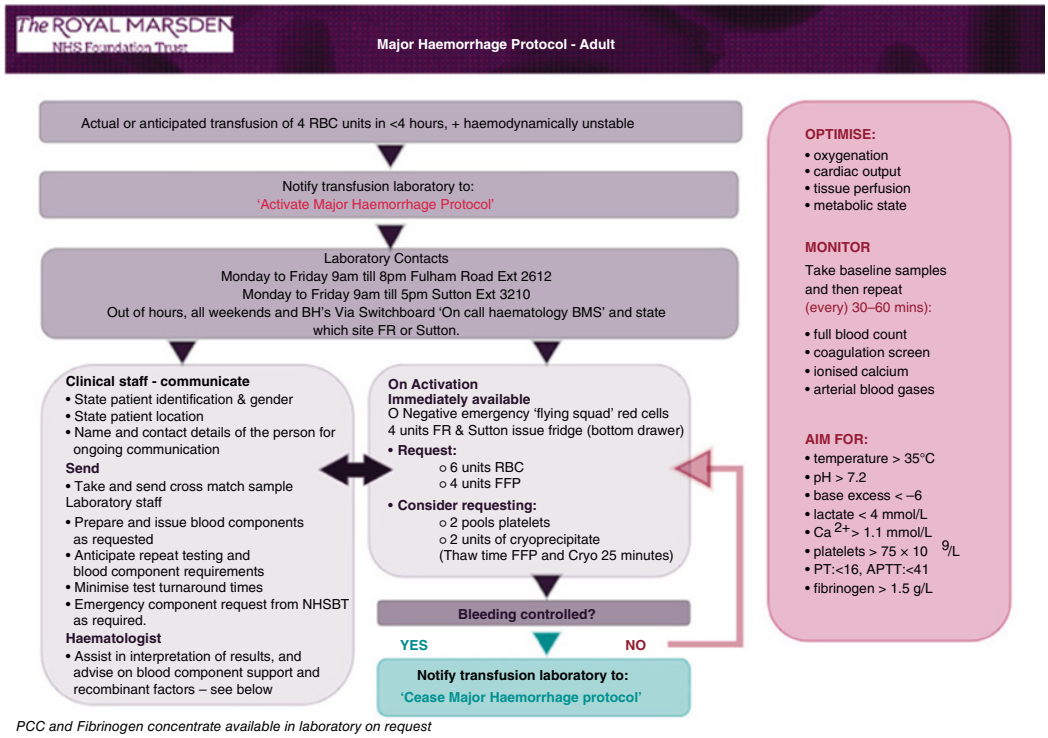

Figure 38.3 Major haemorrhage protocol (Royal Marsden NHS Foundation Trust). APTT, activated partial thromboplastin time; FFP, fresh frozen plasma; PT, prothrombin time; RBC, red blood cell. *Source:* Dougherty 2015. Reproduced with permission of John Wiley & Sons.

provide individualised targeted blood component replacement. National Institute for Health and Care Excellence (NICE) guidelines currently only recommend using VHA-guided algorithms in cardiac surgery, but the value of VHAs in managing major haemorrhage is the subject of current research [38].

Postoperative Measures

Postoperative anaemia is common due to a variety of causes, such as blood losses during surgery, haemodilution, phlebotomy blood loss and decreased erythropoiesis from inflammation and increased hepcidin levels. Efforts to minimise ABT, minimise blood loss and treat anaemia should be continued into the postoperative period.

The use of restrictive transfusion triggers is recommended through the postoperative period in all PBM programmes. The Transfusion Requirements in Critical Care (TRICC) trial demonstrated that a restrictive blood transfusion strategy with a transfusion threshold of < 70 g/: was as effective as a liberal target of < 100 g/L [39]. Meta-analysis showed that these restrictive transfusion thresholds applied across a range of surgical specialties do not adversely impact morbidity and mortality [40]. NICE guidelines recommend a blood transfusion trigger of 80 g/L in patients with acute coronary syndrome, and those recommendations have been extrapolated to patients with cardiac disease [41].

Blood wastage is a major concern, especially with the effects of the COVID-19 pandemic on the availability and use of blood for transfusion [42]. The automatic historical default of two-unit transfusions to achieve an Hb > 10 g/L must be avoided. Choosing Wisely recommends single-unit RBC transfusions for patients who do not have active bleeding [43]. A structured PBM programme includes appropriate transfusion practice, where after each single-unit RBC transfusion haemoglobin levels are measured, the patient is clinically reassessed and further transfusion is given only if necessary. Thus, a single-unit transfusion policy should be the standard of care for blood transfusion practice in all stable patients.

Repeated blood sampling can result in iatrogenic anaemia, particularly in intensive care patients. On average 52.4 mL of blood is withdrawn from each patient on every day they spend in the intensive care unit (ICU) [44]. Reducing the frequency of blood sampling to only essential tests is advisable, and an effort should be made to instil this into a hospital's culture as part of an overarching PBM programme [18]. Additional measures include a reduction in the sample volume by using paediatric blood tubes or using closed inline flush blood-sampling devices for arterial lines to minimise discarded blood waste.

Postoperative anaemia is common. In 152 757 hospitalisations, 72% of patients were discharged with anaemia, and this was associated with severity-dependent increased odds for 30-day hospital readmission compared with those without [45]. The PREVENTT trial raises the question of whether postoperative intravenous iron, before discharge from hospital, may be effective at boosting haemoglobin levels in surgical patients during their recovery period [20]. Indeed, postoperative iron therapy would be easier and less expensive, because the patient would already be in the hospital bed with venous access. Although there is currently limited evidence on postoperative anaemia, there is evidently a high prevalence, which is negatively associated with clinical and long-term outcomes. Further research is required to standardise the measurement and address the true impact of correcting postoperative anaemia on functional and oncological outcomes [46].

Current Challenges and Future Research in Patient Blood Management

PBM aims to improve patient safety and outcomes [47]. In the USA, UK and Europe, PBM has been successfully introduced in some hospitals, and in Australia it has become a

standard of care [48]. Many barriers limit the translation of PBM into clinical practice worldwide. These include clear guidance on clinical pathways, lack of education and training, lack of consensus among key stakeholders in the multidisciplinary team, lack of resources and concerns about risks. It is important to recognise that infrastructure, staff, equipment and economic resources differ considerably between hospitals, thus PBM programmes cannot be a one-size-fits-all approach, but need to be specifically designed for each hospital so they are practical, feasible and institutionally accepted.

Well-implemented PBM programmes can lead to significant cost savings. The benefits of implementing a health system–wide PBM programme were demonstrated in Western Australia, where they saw and associated reduction in mortality, shorter hospital length of stay, less hospital-acquired infection and a stepwise reduction in ABT. An initial investment of US$4.4 million resulted in remarkable cost savings of US$18 million for blood product acquisition costs and US$80 million for reduction of activity-based transfusion costs [49]. A recent meta-analysis has suggested that some PBM interventions (i.e. restrictive transfusion thresholds and TXA) are more cost-effective than others (i.e. presurgery iron administration, cell salvage and point-of-care testing) and could be a good direction for units wishing to start a PBM programme [50].

Future research needs to be directed towards providing higher levels of evidence-based medicine for PBM. These include anaemia management in patients after hospitalisation to enhance recovery, individualised goal-directed haemostatic resuscitation and new clotting concentrates to control massive haemorrhage, optimum transfusion strategy in cardiac patients and understanding of dilutional or traumatic coagulopathy. Research into PBM should focus on patient-related outcomes, not just the now historical focus of blood transfusion.

Conclusion

PBM is an individualised, evidence-based perioperative strategy used to reduce risks and enhance quality of care to improve patient outcomes. These interventions need to be integrated into the pathways patients take from preparation for surgery to postoperative recovery.

Anaemia, bleeding and transfusion are associated with adverse perioperative outcomes. Understanding and investigating anaemia are vital to appropriate management before and after surgery. Haemorrhage management is proactive, and the use of TXA, cell salvage and organised major haemorrhage protocols is essential. Where required, the ABT should be stepwise, a single unit at a time, ideally guided by conventional testing or point-of-care VHAs, to avoid the risks of dilutional coagulopathy and the costs of liberal, unnecessary transfusions.

PBM requires a multidisciplinary team to contribute to this effort through the perioperative period, and strong leaders of PBM are required to embed its concepts and processes into the culture of the hospital. By adopting effective blood management programmes, we can reduce anaemia, bleeding and transfusion in patients undergoing major surgery and improve patient safety and outcomes.

KEY POINTS

1) Hospitals should establish patient blood management (PBM) multidisciplinary teams with PBM pathways, with patient care integrated into anaesthetic and surgical pathways.
2) Preoperative guidelines are necessary for timely anaemia investigation and management, assessment and management of anticoagulant and antiplatelet therapy, strategies for key interventions in patients

with major blood loss, and discussions with patients about the risks, benefits and alternatives for blood transfusions.
3) Operative guidelines are necessary for routine use of tranexamic acid in surgery, cell salvage where blood loss is anticipated to be > 500 mL, and protocols for management of major haemorrhage.
4) Transfusion practice should employ a restrictive transfusion strategy and single-unit transfusion policy in non-bleeding patients.
5) Postoperative care should minimise unnecessary blood tests, recommence necessary anticoagulant and antiplatelet therapy, and consider a long-term anaemia management plan.

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Further Reading

National Blood Authority Australia. Patient blood management guidelines. https://www.blood.gov.au/pbm-guidelines

Network for Advancement of Patient Blood Management, Haemostasis and Thrombosis (NATA). Provides detailed summaries of all PBM techniques with up to date reports of key articles. www.nataonline.com

NHSBT. Patient blood management. https://hospital.blood.co.uk/patient-services/patient-blood-management

Society for the Advancement of Blood Management (SABM). Provides education and research on transfusion medicine. www.sabm.org

39 Restrictive Transfusion Practice and How to Implement It

Marie A. Hollenhorst and Lawrence Tim Goodnough

Departments of Pathology and Medicine, Stanford University, Stanford, CA, USA

Blood transfusion is one of the most frequently performed medical procedures. Guidelines from a number of professional societies recommend restrictive transfusion practice based on high-quality data. Despite this, a substantial proportion of blood products are transfused unnecessarily, subjecting patients to elevated risk of adverse clinical outcomes and increasing the cost of medical care [1].

This chapter begins with a summary of level 1 evidence that supports restrictive transfusion practices, and then discusses the meaning and interpretation of transfusion triggers and targets from trial data. Next, approaches to improving blood utilisation are reviewed, focusing on newer strategies made possible by clinical decision support via electronic health records (EHR).

Level 1 Evidence Supports Restrictive Red Cell Transfusion Practices: Key Clinical Trials

There are a number of randomised controlled trials (RCTs) providing level 1 evidence for restrictive blood transfusion practices [2]. We will focus here on seven key RCTs in adult patients that have compared 'restrictive' versus 'liberal' red cell transfusion strategies in various clinical settings (Table 39.1) [2].

The Transfusion Requirements in Critical Care (TRICC) trial in critical care patients found that randomisation to a restrictive transfusion strategy (haemoglobin [Hb] range 70–90 g/L) had no difference in 30-day mortality rate, when compared to patients transfused more liberally (Hb range 100–120 g/L). The Transfusion Requirements After Cardiac Surgery (TRACS) trial in cardiothoracic (CT) surgery patients was a large, single-centre study with randomisation to receive either restrictive (haematocrit > 24%) or liberal (haematocrit > 30%) red cell transfusions postoperatively. The 30-day all-cause mortality was not different (10% versus 11%, respectively) between the two cohorts. The FOCUS trial in patients undergoing repair of hip fracture found that elderly (mean > 80 years of age) patients were able to tolerate postoperative blood transfusion to an Hb threshold of 80 g/L. It is noteworthy that symptomatic patients in this trial could be transfused at higher Hb levels. A study of patients with upper gastrointestinal (UGI) bleeding found that patients randomised to a restrictive (< 70 g/L) versus a liberal (< 90 g/L) Hb threshold for blood transfusions actually had statistically significantly

Practical Transfusion Medicine, Sixth Edition. Edited by Michael F. Murphy, David J. Roberts, Mark H. Yazer and Nancy M. Dunbar.

Table 39.1 Seven key clinical trials of blood transfusion in adults.

Study	Clinical setting	Haemoglobin threshold (g/L)	Age (years)	Patients transfused (%)	Deviation from protocol (%)	Mean haemoglobin (g/L)	% of screened patients enrolled in study
TRICC[a]	Intensive care	70 100	57.1 58.1	67 99	1.4 4.3	85 107	41
TRACS[b]	CT surgery	80 100	58.6 60.7	47 78	1.6 0.0	91 105	75
FOCUS[c]	Hip fracture repair	80 100	81.5 81.8	41 97	9.0 5.6	79 92	56
Villaneuva[d]	Acute upper GI bleeding	70 90	NA NA	49 86	9.0 3.0	73 80	93
MINT Pilot[e]	Symptomatic CAD	80 100	74.3 67.3	28.3 NA	1.8 9.1	79 93	12.2
TRISS[f]	Septic shock	70 90	67 67	64 99	5.9 2.2	77 93	82
TITRe2[g]	Cardiac surgery	75 90	69.9 70.8	53.4 92.2	30 45	80–90 92–98	98

CAD, coronary artery surgery; CT, cardiothoracic; GI, gastrointestinal; NA, not available.
[a] NEJM 1999;**340**:409–17.
[b] JAMA 2010;**304**:1559–67.
[c] NEJM 2011;**365**:2453–62.
[d] NEJM 2013;**368**:11–21.
[e] Am Heart J 2013;**165**:964–71.
[f] NEJM 2014;**371**:1381–91.
[g] NEJM 2015;**372**:997–1008.
Source: Adapted from Goodnough & Shah 2015 [2].

improved clinical patient outcomes, including 45-day mortality and rebleeding rates.

Patients who have had a myocardial infarction have generally not been well represented in studies of red blood cell (RBC) transfusion thresholds. Results from the Myocardial Ischemia and Transfusion (MINT) pilot trial suggested that a liberal transfusion threshold (Hb ≥ 100 g/L) may be superior to a restrictive threshold (Hb < 80 g/L) in patients with acute coronary syndrome or stable angina. This hypothesis is being tested now in the definitive MINT trial, which is enrolling anaemic patients with myocardial infarction at multiple centres.

A trial in patients with septic shock of lower (< 70 g/L) versus higher (< 90 g/L) Hb thresholds for red cell transfusion found equivalent 90-day mortalities (43% vs 45%, respectively) in the two patient cohorts. Finally, the TITRe2 trial studied elective cardiac surgery patients randomised to single-unit blood transfusions at a restrictive Hb threshold of 75 g/L or a liberal Hb threshold of 90 g/L. There was no significant difference in the primary composite outcome of serious infectious (sepsis or wound infections) or ischaemic events (stroke, myocardial infarction, ischaemic bowel and acute renal injury) between these two groups. While only 53% versus 92% of patients received blood transfusions in the restrictive and liberal cohorts, there was no difference in the primary outcome (35.1% and 33.0%, respectively), nor was 30-day mortality different. However, the average daily Hb levels were not that far apart (80–90 g/L vs 92–98 g/L); moreover, deviations from the protocol occurred in 30% and 45%, respectively, of the restrictive versus liberal threshold cohorts, perhaps accounting for the inability to demonstrate a predicted difference in the primary outcome. The authors conducted a *post hoc* analysis of 90-day mortality that was higher in the restrictive group, compared to the liberal group (4.2% vs 2.6%, p = 0.045).

A Cochrane systematic review of prospective, randomised trials up to 2016 compared 'higher' versus 'lower' Hb triggers for blood transfusion in 31 trials involving 12 587 patients. The authors found that lower Hb thresholds were as well tolerated as higher Hb levels. Blood transfusions could be reduced by 43% in cohorts using a lower Hb threshold for transfusion [3].

Transfusion Triggers and Targets

The goal of RBC transfusion is most commonly to improve end-organ oxygen delivery. In clinical practice, end-organ oxygen delivery is difficult or impossible to measure, so as a proxy RBCs are often transfused based on laboratory test results. The Hb trigger is defined as the lowest Hb concentration that will be tolerated before transfusion therapy is initiated. While Hb is a useful metric and in many situations correlates with end-organ oxygen delivery, the ideal Hb trigger will vary depending on the patient and clinical situation. In the case of rapid acute blood loss, Hb will poorly reflect the degree of anaemia. Furthermore, Hb is affected not just by loss of RBC mass, but also by changes in the plasma volume. Finally, Hb laboratory triggers do not take into consideration individual patient adaptations that can occur to chronic anaemia, which allow some patients to tolerate substantially lower Hb than other patients.

The clinical trial data described above has generally been interpreted as supporting a restrictive strategy with a Hb trigger of 70 g/L in most patients [4]. However, it is important to consider not simply the Hb triggers under study in the randomised trials, but also the Hb targets. The Hb target is the desired Hb value after the transfusion, and will depend on several variables, including (1) Hb trigger, (2) rate of loss or haemolysis of RBCs, (3) rate of haemoconcentration or haemodilution, (4) blood volume and (5) number of units of RBCs transfused. Figure 39.1 shows that the average target Hb in these trials is often 10–15 g/L higher than the Hb trigger that defines the transfusion strategy. The FOCUS trial, for example, compared Hb

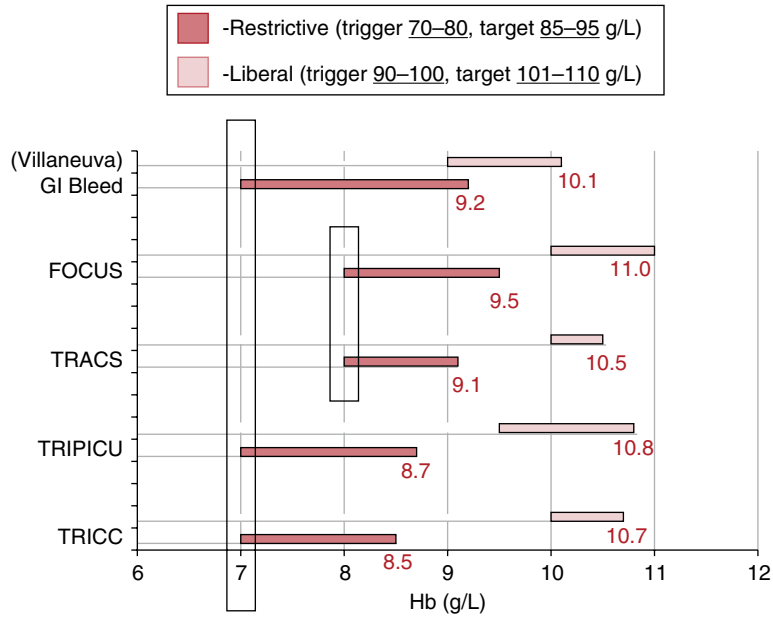

Figure 39.1 Haemoglobin (Hb) triggers (Hb before transfusion) and Hb targets (Hb after transfusion) are shown from five large randomised trials that compared restrictive and liberal transfusion strategies. The trial results advocate for the use of a restrictive transfusion strategy, with Hb triggers of 70–80 g/L for most patients. The Hb target concentration in the restrictive group was 85–95 g/L. When this finding is considered in the Functional Outcomes in Cardiovascular Patients Undergoing Surgical Hip Fracture Repair (FOCUS) trial, for example, the two groups compared (Hb trigger 80 vs 100 g/L) had actual daily average Hb concentrations that were 95 g/L in the restrictive group and 110 g/L in the liberal group.

triggers of 80 and 100 g/L, but the two groups ended up with Hb target concentrations of 95 and 110 g/L, respectively [4].

Clinical Practice Guidelines

A number of published clinical practice guidelines for RBC transfusion are available (Table 39.2) [5]. It is generally agreed that transfusion is not of benefit when the Hb is greater than 100 g/L for most patients, but may be beneficial when the Hb is less than 60–80 g/L. While the Hb trigger thresholds in these guidelines can be useful to guide transfusion decisions, the decision to transfuse should never be based solely on a laboratory value. The clinical and laboratory features of each case must be synthesised, in concert with available clinical trial data, in order to make an individualised decision regarding transfusion in each case. Factors such as the chronicity of the anaemia, presence or absence of anaemia-related symptoms, and co-morbid conditions such as cardiac ischaemia are all important, and make it impossible to codify a single Hb trigger that is appropriate for all patients.

Strategies for Improving Blood Utilisation

As described in other chapters of this textbook, an integrated patient blood management programme includes a toolkit of strategies to ensure optimal patient outcomes while minimising overuse of blood products. Key facets include strategies to optimise haematopoiesis and minimise blood loss. The remainder of this chapter will discuss strategies to support use of optimal

Table 39.2 Clinical practice guidelines for red blood cell transfusion for hospitalised adult patients.

Year	Society	Recommended haemoglobin (Hb) trigger for transfusion (g/L)	Reference
2011	Society of Thoracic Surgeons	< 60–70	Ann Thorac Surg 2011;**91**:944–82
2011	SABM	< 80	Trans Med Rev 2011;**25**:232–46
2012	British Committee for Standards in Haematology	< 70, critically ill patients	Br J Haematol 2012;**160**:445–64
2012	Australian National Blood Authority	< 70 likely to be appropriate, > 100 usually inappropriate	https://www.blood.gov.au/system/files/documents/20180424-PBM-Module3.pdf
2012	KDIGO	No number	Kid Int 2012;**2**:311–16
2013	American College of Physicians	< 70–80 in coronary heart disease patients	Ann Intern Med 2013;**159**(11):770
2014	British Committee for Standards in Haematology	No number	Br J Haematol 2014;**164**:503–25
2015	National Institute for Health and Care Excellence (NICE)	< 70 for most patients < 80–100 for patients with acute coronary syndrome	https://www.nice.org.uk/guidance/ng24
2015	American Society of Anesthesiologists	No number	Anesthesiology 2015;**122**:2:241–75
2016	AABB	70–80 or 80*	Transfusion 2016;**56**:2627–30
2019	Frankfurt Consensus Conference	< 70 for critically ill < 75 for cardiac surgery < 80 for hip fracture and cardiovascular disease or risk factors < 70–80 for acute gastrointestinal bleed	JAMA 2019;**321**(10):983–97

* For patients with symptoms of end-organ ischaemia.
KDIGO, Kidney Dialysis Improvement Global Outcomes.
Source: Adapted from Goodnough 2013 [6].

transfusion practice in patients for whom a blood transfusion is being considered.

Despite the available high-quality data and clinical guidelines that support use of a restrictive RBC transfusion practice, RBC transfusion remains one of the most commonly overused procedures in medicine. A variety of approaches have been taken in an effort to encourage appropriate utilisation of blood transfusions. Such approaches include educational initiatives, retrospective utilisation review and concurrent order review. Such interventions can be resource intensive and difficult to sustain in the long term.

Clinical Decision Support

As use of EHRs has increased, transfusion medicine specialists have recognised the potential benefits of leveraging electronic ordering systems to improve blood utilisation. Clinical decision support (CDS) describes the use of an electronic system to provide treatment recommendations based on characteristics of an individual patient and local guidelines.

A number of studies have evaluated the effects of CDS on blood-ordering practices. CDS has been most studied in the context of RBC transfusion. Reported CDS interventions for promotion of restrictive RBC transfusion have varied in complexity, from simple alerts triggered by an order for RBC transfusion if the most recent Hb value exceeds a set threshold, to more complex systems that trigger at variable Hb values depending on the indication for transfusion or patient-specific factors.

The authors of a recent systematic review of 20 studies came to the conclusion that there is good evidence that CDS can lead to improved adherence to RBC transfusion guidelines [7]. All of the studies that reported financial outcomes described cost savings, generally of the order of tens to hundreds of thousands of dollars of annual savings per year. The authors of this review noted that there was not a convincing effect of CDS implementation on patient outcomes or on use of blood products aside from RBCs. A major limitation of CDS studies is the almost uniform before–after trial design, without any element of randomisation. This leaves open the possibility that factors other than CDS implementation could have been responsible for any changes in blood product ordering between the two study periods.

Design elements leading to effective CDS have been published. A review of CDS found that 68% improved clinical practice and, in 32 systems that possessed four key features, 94% significantly improved clinical practices [8]. These four key features can be summarised as computer-based decision support that provides recommendations (rather than assessments) automatically at time of physician order entry (POE), through a normal workflow. Subsequent analyses highlighted some unique aspects contributing to success: presenting CDS to both providers and patients, involving local users in the development process and not requiring additional data entry by providers. A required override or acknowledgement reason from providers increases adherence to CDS recommendations.

Overall, there is not generalisability in CDS. CDS parameters should be designed appropriately to meet the clinical and business needs, along with the right exclusions to avoid alert fatigue and false-positive alerting. These parameters include the audience (physician vs pharmacist vs nurse), the setting (inpatient, outpatient, operating areas) and mode of alerting, such as passive (information pulled by users), semi-active (knowledge representation and call to action) or active (automatic action without user intervention or knowledge).

One analysis found facilitators and barriers to CDS in three broad categories: user performance expectancy of system, user effort expectancy and social/cultural factors [9]. Key themes within barriers included provider lack of time/competing clinical duties, poor design (complex or unfriendly interface, lack of agreement with system, lack of

awareness of content) and cultural factors (reluctance to use CDS in front of patients, poor computer skills, financial constraints and low social acceptance of CDS). This analysis provides a road map for the future design and redesign of CDS to better engage end users to achieve more meaningful clinical impact. Clinician engagement is important for both the build and acceptance of these systems, but the resources supplied to physicians to meet these demands or financial incentives to drive these recommendations are absent.

Improving Blood Utilisation: The Stanford Experience

At Stanford Health Care (SHC), we implemented a CDS system (Figure 39.2) for ordering blood transfusion in which a smart best practice alert (BPA) is triggered when a clinician orders blood for a patient whose pretransfusion Hb is above a threshold (70 g/L, or 80 g/L for patients with acute coronary syndrome or post cardiothoracic procedure) [10,11]. The purpose of the Hb level is to serve as a threshold for triggering concurrent utilisation self-review (by the ordering medical team) and provide links to published medical literature. The BPA does not identify a 'correct' Hb level, as even the most intelligent design cannot capture all relevant clinical and laboratory parameters. Nevertheless, since implementation of the BPA for red cell transfusion in July 2010, the percentage of transfusions in patients whose pretransfusion Hb was greater than 80 g/L decreased from 60% beforehand to 35% in the six months after implementation, with a continued downtrend to less than 30% through 2013 ($p < 0.001$) [12].

Overall, following implementation of CDS, red cell transfusions were reduced by 42% from 2009 to 2015 (Figure 39.3), despite increases in patient days at risk and case-mix complexity over this time period. Hospital-wide patient clinical outcomes, as demonstrated by mortality, showed statistically significant improvement, while length of stay and 30-day readmission rates remained stable. Outcomes in the subcohort of transfused patients before and after implementation of CDS showed pronounced improvement ($p < 0.01$ for all three outcomes) [12]. While the improvement in patient outcomes concurrent with reduction in red cell transfusions cannot be proven to be causal, it is reassuring that there was no deleterious effect on patient outcomes after hospital-wide adoption of restrictive transfusion practices, particularly in view of lower discharge Hb levels [13]. A study monitoring for inappropriate undertransfusion found no evidence that cases of non-administration of blood were unjustified [14].

Additional benefits of our restrictive transfusion strategy included a significant improvement in the laboratory budget, with net annual savings of $1.6 million (£1.3 million) [11]. Purchase acquisition costs represent a fraction of total costs of blood transfusion that additionally include laboratory testing, reagent costs, nursing time dedicated to transfusion and monitoring. An activity-based cost summary of blood transfusions estimates that total costs related to transfusion are 3.2–4.8 times the purchase costs. Hence, the total transfusion-related saving potentially surpasses $30 million, over a four-year period. We subsequently implemented a smart BPA for plasma, triggered when the last recorded international normalised ratio (INR) is ≤ 1.7, to guide more appropriate plasma transfusion; following implementation there was a 17% reduction in plasma utilisation [15].

This model of concurrent real-time utilisation review can be supplemented by benchmarking between service lines and between providers in a service line for appropriateness of transfusion. Since up to 30% of red cell transfusions continue to occur in patients whose Hb was greater than 80 g/L at our institution, benchmarking can increase stakeholder acceptance, help reduce variability between providers and/or help modify the CDS triggers for known clinical exceptions. This process serves as

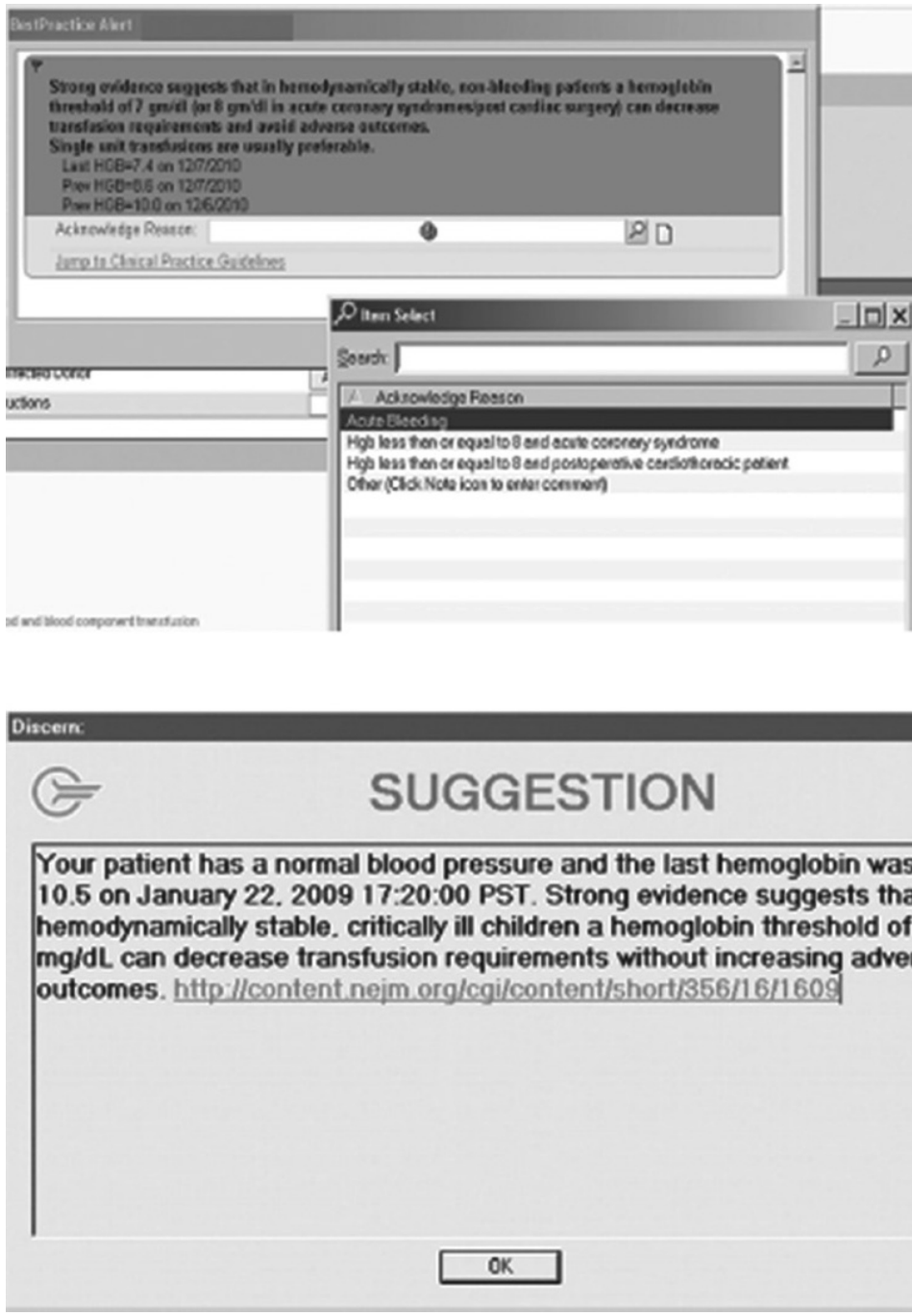

Figure 39.2 Best practice alert (BPA) screenshots at Stanford University Medical Center (SUMC). Screenshot from an electronic physician order entry (POE) for blood transfusion in adult patients at Stanford Hospital and Clinics (SHC) illustrates an 'interruptive alert' as a reminder for the merits of a restrictive transfusion practice versus liberal transfusion practice. An acknowledgement/exception field allows the physician to provide the indication for transfusion (acute bleeding, haemoglobin < 80 g/L in the acute coronary syndrome or post-cardiothoracic surgery patient, other clinical scenario) if such clinical scenarios were not updated in the problem list. The BPA for paediatric patients at Lucille Packard's Children's Hospital (LPCH) triggers only for children ages 1–18 years with haemoglobin > 70 g/L who are normotensive in the last six hours. The alert does not trigger in patients from cardiac, haematology-oncology and neonatal intensive care unit (ICU) wards. Note that the text in the 'Suggestion' box has a typo: haemoglobin concentration should be listed as 7 gm/dL not 7 mg/dL. *Source*: Goodnough and Shah [10]. Reproduced with permission of Oxford University Press.

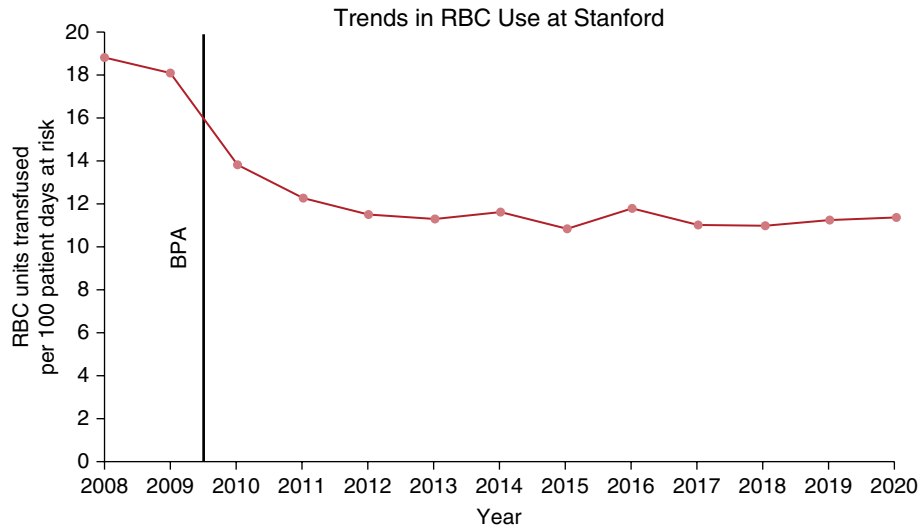

Figure 39.3 Trends in blood utilisation. Blood components issued to patients at Stanford Health Care. Transfusion of red blood cells (RBCs) decreased by 24% from 2009 to 2020. *Source*: Adapted from Goodnough et al. 2016, 2019 [16,17].

continuous education and feedback, which is seen as vital in the success of utilisation programmes by augmenting improvements through CDS.

Other programmes have been able to utilise EHRs to improve blood utilisation in a different manner. One approach is to reconfigure the computerised POE system for non-bleeding (excluding procedural units such as operating rooms, cardiac catheterisation labs) patients to remove single-click ordering for two-unit red cell transfusions; the provider must select from a drop-down menu if additional red cell units are desired. The proportion of two-unit red cell transfusions at one centre decreased from 47% before to 15% after this intervention [18]. Similarly, reductions in two-unit red cell orders (48% to 33%) and an increase in one-unit red cell transfusions (22% to 48%) had been found after a comprehensive education and audit programme promoting restrictive transfusion practices [19]. While CDS can improve red cell usage, further data are needed to assess whether CDS can improve plasma and platelet utilisation.

Future Directions

According to the United States Centers for Medicare and Medicaid Services, in 2019 $3.8 trillion (£2.8 trillion) was spent on healthcare, consuming 17.7% of gross domestic product (GDP). Almost a third of this healthcare expenditure is estimated to be wasteful. Reducing this waste not only helps to improve patient outcomes by reducing exposure to unnecessary treatments/tests, but also addresses patient concerns about the high cost of healthcare. Blood transfusion has been targeted among five key overuse measures by the American Board of Internal Medicine. The increased adoption of EHRs and features such as alerting allows the practice of prospective, real-time monitoring of transfusion therapy in an automated fashion at the critical time of POE.

While alerting can help conform clinical practice to published guidelines, this electronic tool may often be ignored if it is implemented in a vacuum without concurrent education. The contributions of the individual elements of the CDS (alert, link to

relevant literature, acknowledgement/exception) need to be further analysed to understand components affecting end-user action. Frequent and ill-designed CDS exacerbates 'alert/click fatigue', where users begin to mechanistically cancel pop-ups without reading the message, particularly when these occur at the time of order signing. Future measures include providing the prescriber with evidence-based and practical blood product ordering options; limiting low-impact CDS; moving the decision making and support downstream to Pharmacy, Radiology or Pathology; and distributing the CDS burden to personnel with the highest knowledge base to make a decision. Displaying recommendations alongside user search items at order entry, instead of at the time of signing, would prevent disruptions in workflow and may lead to higher acceptance of CDS. An additional item missing from many CDS is follow-up of users and groups/services that commonly disregard best practices. Long term, these groups will have to be engaged for further education or refinement of CDS for continuous quality improvement.

Conclusion

Inappropriate blood transfusions and related costs can be effectively addressed through real-time CDS. Clinical patient outcomes improve after implementation; these observations provide assurance that a restrictive transfusion strategy can be successfully implemented institution-wide, to improve patient safety and avoid patient harm. CDS design will require continued maturation to optimise user engagement and end-action while minimising 'alert fatigue'. In deriving increased value out of healthcare, CDS also can be applied to other overuse measures in laboratory testing, radiology and other therapies such as antibiotics, as outlined by the ABIM Choosing Wisely Campaign.

KEY POINTS

1) Blood transfusion is one of the most common inappropriately ordered therapies.
2) High-quality clinical trial data support restrictive red blood cell transfusion practice in most situations.
3) It is important to consider not only the haemoglobin (Hb) trigger (prior to transfusion) but also the Hb target (post transfusion). The Hb target is usually 10–15 g/L higher than the trigger in the randomised controlled trials that support a restrictive transfusion strategy.
4) Clinical decision support is an important tool for improving red blood cell utilisation.
5) Further studies of clinical decision support approaches to improving utilisation of blood products other than red blood cells are needed.

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40 Using Data to Support Patient Blood Management

Nadia B. Hensley[1], Sandaruwani Abeysiri[2] and Steven M. Frank[1]

[1] *Department of Anesthesiology/Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD, USA*
[2] *The Royal Marsden NHS Foundation Trust, London, UK*

> Even with recent advances in electronic medical records, it is entirely possible that many industries have better automated data collection than hospitals.
>
> *(Steven M. Frank)*

Patient blood management (PBM) is a relatively recent development in medicine that, in simple terms, has been described as giving the right product, at the right dose, at the right time, to the right patient, for the right reason. Although a strong PBM programme is multifaceted in its approach, all aspects of running a PBM programme are data intensive. Prior to the 1960s most patient records were kept in paper form, but as technology began improving, rudimentary local electronic medical record (EMR) systems were being set up locally. Then in the 1970s the American Federal Government commenced the use of EMR systems with its Department of Veteran Affairs, which continued to expand through the 1990s. In spite of this progress, in 2013 it was determined that more than half of US hospitals were still using paper anaesthesia records [1]. Given the difficulty in analysing data from paper records, the EMR is a big step forward. One problem with EMRs is that they are primarily designed for putting data in and not for getting data out, and their focus is often on billing. Once a process is developed for data extraction, the EMR will be a goldmine of data that can be used to improve clinical practice.

Although the kinds of data in the EMR are numerous, the most important variables used to support a PBM programme are listed in Table 40.1. Data for the entire hospital can show changes in overall blood utilisation, which can then be narrowed down to the department level (specialty service), and then down to the individual providers. Provider-level data can be challenging to interpret owing to problems with attribution. For example, in the operating room the decision to transfuse may come from either the surgeon or the anaesthesiologist, and likewise in the intensive care unit (ICU) the decision may come from either the intensivist or the primary attending physician. Each facility should come to an agreement over how to handle these attribution challenges. Some institutions have mandated that an authorising physician be indicated in the electronic order set for each transfusion order, to address the attribution problem.

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Table 40.1 Data variables used to support patient blood management (PBM).

Parameter measured	Data variable	Units of measure	Implications
Blood utilisation	Average number of units/hospital inpatient	Mean units/admitted patient	Best method to measure success of a PBM programme Has direct implications for cost savings Is inherently adjusted by patient volume
	Percentage of inpatients transfused	Percentage of patients	Useful information on trends for transfusion avoidance
	Total units transfused	Units per month or year	Useful to assess overall blood use and costs, but is not patient volume adjusted Can be used to include outpatient transfusions
Transfusion guideline compliance	Transfusion triggers	Percentage of transfusion orders placed outside of evidence-based guidelines assessed by laboratory tests	Can be assessed by 'true trigger' (most recent lab value before transfusion), or by nadir haemoglobin during hospital stay
	Transfusion targets	Percentage of transfused patients with discharge laboratory values outside evidence-based range, as assessed by laboratory tests	Can be assessed by posttransfusion laboratory values, but more easily assessed by last laboratory value before discharge
Clinical outcomes	Incidence of morbid events	Percentage of patients with morbid event (e.g. ischaemic, thrombotic, infection, renal or respiratory; see Table 40.2)	Useful to determine whether PBM efforts affect quality of care
	Incidence of mortality	Percentage of patients not surviving hospital stay	Useful to determine whether PBM efforts affect mortality
	Length of stay (LOS)	Days – best analysed as median (interquartile range), because LOS not normally distributed	Has direct implications for cost of care; transfusion is associated with increased LOS
Costs	Hospital costs	$/hospitalisation Can be subdivided into direct and indirect costs	Useful because costs of blood and overall patient care are both included
	Hospital charges	$/hospitalisation	Less useful, because hospitals are not always paid what they change
Risk-adjustment variables	Case-mix index APR-DRG Wt MS-DRG Wt	 0–30-point scale 0–30-point scale	Takes into account both severity of illness and complexity of procedures; used for Medicare billing and reimbursement; is directly related to transfusion requirements
	Charlson Comorbidity Index	0, 1–2, 3–4 and ≥ 5 scores are directly related to 1-year and 10-year mortality	A weighted index that takes into account co-morbidities; may correlate with transfusion requirements

APR-DRG Wt, Weighted All Patient Refined Diagnosis-Related Group; MS-DRG Wt, Weighted Medicare Severity Diagnosis-Related Group.

Transfusion Triggers and Targets

Because end-organ oxygen delivery is difficult or impossible to measure, the indication for transfusion is most often based on laboratory test results. The haemoglobin (Hb) trigger, for example, is defined as the lowest Hb concentration that will be tolerated before transfusion therapy is initiated. This method of determining the need for red blood cell (RBC) transfusion is very useful, especially when the patient has adequate intravascular volume and is not actively bleeding. Over the past decade, 11 large randomised trials have compared a restrictive transfusion strategy (Hb trigger of 7–8 g/dL) to a liberal strategy (Hb trigger of 9–10 g/dL) to determine differences in clinical outcomes. In general, the findings support the use of a restrictive strategy, with a trigger of 7 g/dL in most patients, and 7.5–8 g/dL in those with cardiovascular disease. The first of these studies, the TRICC trial [2], showed that even the sickest patients in the hospital (ICU patients with a 25% overall mortality rate) did no better with a 10 g/dL trigger than with a 7 g/dL trigger. What has been largely ignored in these large trials, however, is the average daily Hb concentration in the restrictive and liberal groups after the triggers are put into practice. If the trigger represents the Hb threshold for initiating transfusion, the 'Hb target' is the Hb value after the transfusion [3].

The Hb target is also a means to assess the dose of blood given. Even with a perfect evidence-based, restrictive Hb transfusion trigger, if a large dose of blood is administered, the Hb target will exceed the evidence-based range. By encouraging single-unit RBC transfusions, practitioners can avoid exceeding the Hb target and conserve blood. The problem is that one must read the randomised trial publications carefully to determine what Hb target was actually achieved in the liberal and restrictive groups. Figure 40.1

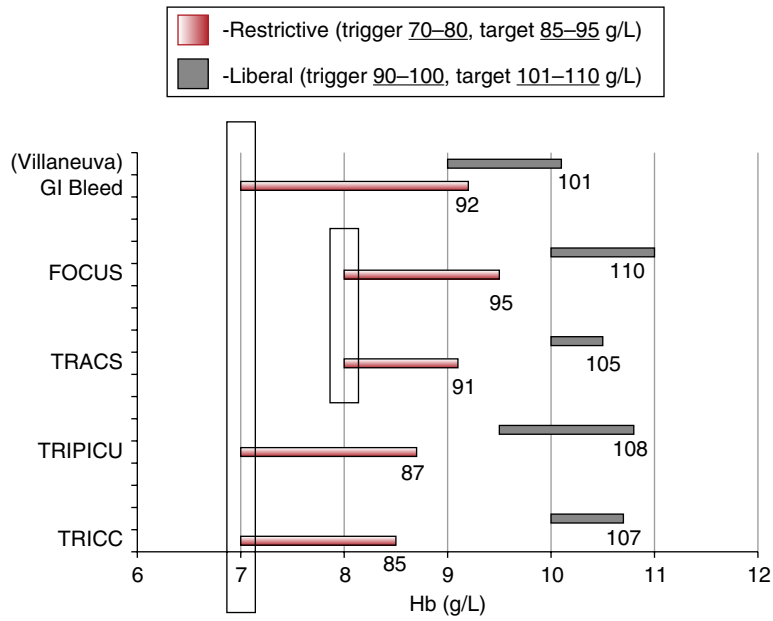

Figure 40.1 Haemoglobin (Hb) triggers (Hb before transfusion) and Hb targets (Hb after transfusion) are shown from five of the early large randomised trials [2,4–7] that compared restrictive and liberal transfusion strategies. The left edge of the bars is the Hb trigger and the right edge of the bars is the Hb target. Although the trial results advocate for the use of a restrictive transfusion strategy, with Hb triggers of 7–8 g/dL for most patients, the Hb target concentration in the restrictive group was 8.5–9.5 g/dL. When this finding is considered in the FOCUS trial, for example, the two groups compared (Hb trigger 8 vs 10 g/dL) had actual daily average Hb concentrations that were 9.5 g/dL in the restrictive group and 11.0 g/dL in the liberal group.

illustrates these findings in five of the earlier trials, and clearly shows an average Hb target that is often 1–1.5 g/dL higher than the Hb trigger that defines the transfusion strategy. The FOCUS trial [4], for example, compared Hb triggers of 8 and 10 g/dL, but the two groups were transfused to Hb target concentrations of 9.5 and 11.0 g/dL, respectively. In reality, we treat patients, not just laboratory values, so other factors like symptoms of anaemia, active bleeding and intravascular volume should be part of the transfusion decision process.

Because it is often difficult to collect accurate data on pre- and posttransfusion Hb levels, especially when patients receive multiple transfusions, some have advocated using trigger and target surrogate values that are easier to collect and analyse. The nadir Hb level for an entire hospital stay has been used as a surrogate for the Hb trigger, and the last Hb measured before discharge can be used as a surrogate for the Hb target [3]. In reality, both the real trigger (Hb measured before transfusion) and the surrogate trigger could be misleading if the laboratory tests were not ordered, for example, during a bleeding episode. The same methods can be used for the international normalised ratio (INR) and plasma transfusions, or for platelet counts and platelet transfusions. However, this approach is limited, because evidence is scant for bleeding outcomes and triggers based on laboratory tests alone for these blood components.

Blood Utilisation Metrics

Besides triggers and targets, other metrics commonly used to assess blood utilisation include the following (see Table 40.1):

- Average number of units transfused per patient. This measure is calculated by dividing all transfused units by all admitted patients. It has been used as the classic definition of 'blood utilisation' [8], perhaps because it correlates most closely with overall transfusion costs when adjusted for a facility's patient volume. This metric is probably the most useful method of tracking the success of a PBM programme. However, when used to compare providers to their peers, it is only useful if all providers are performing similar procedures on similar patients [9].
- Average number of units per transfused patient. This measure is calculated by dividing the number of all transfused units by the number of all transfused patients. Although it is used in some PBM programmes, it is less useful than the previous measure because it ignores all patients who avoid transfusion, often as a result of successful blood conservation measures.
- Percentage of patients transfused. This measure is calculated by dividing the number of patients transfused by the total number of hospitalised patients. As with the first measure, this metric is most useful for comparing providers to their peers when they are all performing similar procedures in similar patients. However, it is less useful than the average number of units transfused per patient because, regardless of whether a given patient receives one or ten units, they count as only one transfused patient.

Sources of Data

Although it is possible to collect data from paper medical records, the process is arduous and useful only for random sampling of transfusion guideline compliance. Now with the EMR data are plentiful, but extraction can be challenging. A dedicated data manager with expertise in relational database management is ideal for extracting, analysing and preparing dashboards and reports to support PBM. The cost of putting in place an automated system for reporting blood utilisation can be substantial, and the data manager must continually monitor the system to keep pace with changes in the underlying

databases and security requirements. Therefore, some institutions outsource this process. Specific EMRs offer their own reporting tools, which require additional training, and sometimes an institution has multiple different computer systems and databases, making data extraction challenging.

Another data source is the anaesthesia information management system [10], which is the term for the electronic anaesthesia records. The limitation of this data source is that only the intraoperative period is covered. We were surprised to learn that only about one-sixth of all transfused units are administered in the operating room at our institution (Johns Hopkins Hospital, Baltimore, MD, USA). In addition, Hb transfusion triggers are often and appropriately higher during surgery, when many patients are actively bleeding.

With many institutions adopting the EMR from Epic (Verona, WI, USA), extraction of data directly from the Epic database has become commonplace. This requires a certified Epic Report Writer, who receives training from the Epic headquarters, allowing the individual to extract data from the EMR, which are stored on Microsoft (Redmond, WA, USA) SQL servers. Using such data, along with a business intelligence platform (e.g. Qlikview [King of Prussia, PA, USA] or Tableau [Seattle, WA, USA]), we have created blood management dashboards [11] for our six-hospital health system, allowing us to analyse and present most of the variables listed in Table 40.1, and to export such data in a spreadsheet format, allowing the use of any chosen software package.

These dashboards can be used to assess high-level data for the entire health system, or to assess individual hospitals or departments, or even to drill down to individual providers. On our dashboards, it was easy to identify a decrease in double-unit RBC transfusion orders (from 40% to 15% of all RBC orders), and a decrease in RBC orders placed with a preceding Hb > 8 g/dL (from 50% to 21%), for the Johns Hopkins Health System as a result of our PBM programme.

Data Extraction, Analysis and Presentation to Improve Practice

Extracting data from the EMR is often the hardest task. The data may be scattered across databases and across units within an institution. This lack of centrality introduces administrative and technical barriers. Extracting and merging these data requires specialised skills, such as structured query language (SQL) programming. The simplest form of extracted data is a 'flat file', with one row per item of interest (e.g. patient admission). This format entails loss or aggregation of information, such as multiple surgical events per admission. For a typical analysis, the extracted data are imported into an application, which may be a spreadsheet program, but more sophisticated tools may be needed, such as those provided by SAS or JMP (both from SAS Institutes, Cary, NC, USA) or Stata (StataCorp, College Station, TX, USA). One useful application now is 'R' (http://www.r-project.org), a versatile statistical programming language that dates back to 1984 and is free to download. It has a steeper learning curve than other choices, but it has thousands of contributed add-on libraries for any imaginable data analysis or presentation task.

In our experience, the most effective method of showing data to providers is the rank-order bar graph (Figure 40.2). When providers are compared directly to their peers [10], usually within their own specialty service, the individuals transfusing more blood, or to higher Hb values outside the evidence-based range, will want to change their practice to be closer to the mid-range on the graph. It is debated whether to present such data with physician codes or with their names; however, the most impact occurs when names accompany the data. One lesson we have learned is that comparing surgeons by average Hb triggers and targets is better received than comparing surgeons by percentage of patients transfused, or by average number of units transfused per patient. To give an example, surgeon #44 (in Figure 40.2), with the highest average Hb

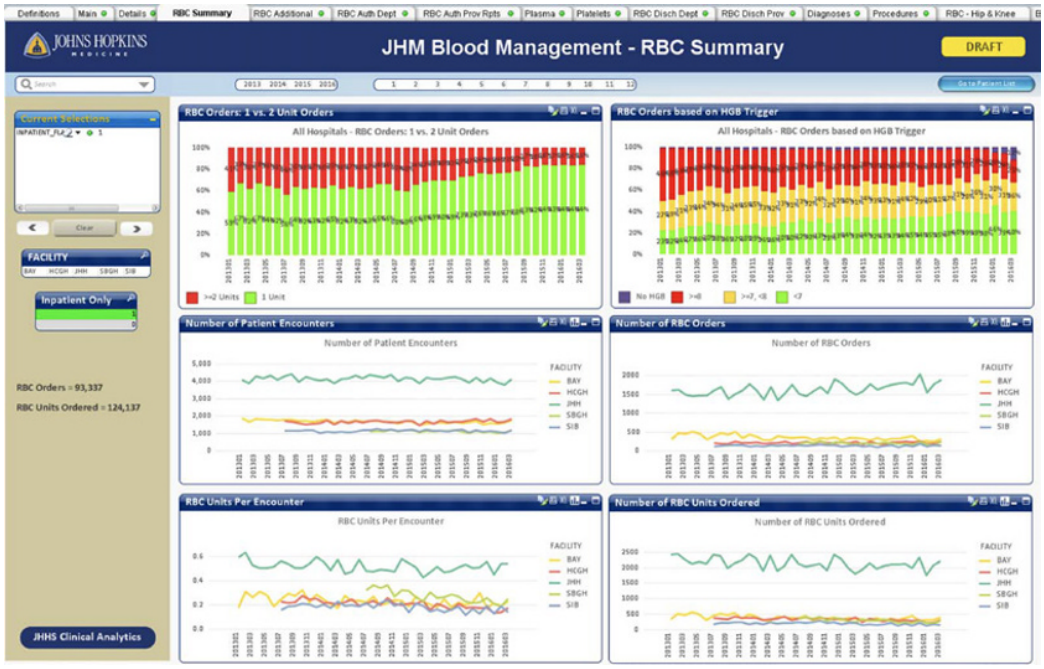

Figure 40.2 Comparison of mean transfusion haemoglobin (Hb) triggers and targets for all surgeons and anaesthesiologists who had 10 or more patients in the anaesthesia information management system (AIMS) database. The mean Hb triggers are designated by the left edge of the red bars and the mean Hb targets by the right edge of the red bars. The span between the lowest and highest Hb triggers was 2.6 g/dL for surgeons and 2.4 g/dL for anaesthesiologists. The span between lowest and highest Hb targets was 3.0 g/dL for surgeons and 2.8 g/dL for anaesthesiologists. *Source*: Wintermeyer et al. 2016 [11].

trigger and target, had no idea where he stood on the Hb curve until we showed him the data. Afterwards, his blood utilisation in average units per patient decreased by more than 60%. We have used similar methods for comparing anaesthesiologists to their peers using rank-order bar graphs, showing percentage of patients transfused and average number of blood units per patient, for cardiac surgery [12]. By displaying these graphs on the doctor's office door (Figure 40.3), there was a great level of interest to see where one stood on the transfusion rankings, which resulted in improved blood utilisation. In effect, such audits are akin to a psychology experiment, resulting in behavioural change.

Perhaps the most effective method of presenting transfusion data is shown in Figure 40.4. We obtained the most recent measured pretransfusion Hb level (Hb trigger) by comparing the EMR time stamps of the laboratory tests and the transfusion orders. If no Hb test had been ordered within 24 hours before transfusion, then the trigger was considered to be missing. The various attending physicians in Figure 40.4 are shown in rank order by the number of RBC orders placed over the selected period. The proportion of RBC orders is according to the Hb trigger levels: green shows the percentage of orders with a trigger < 7 g/dL; yellow, a trigger of 7–7.9 g/dL; and red, a trigger ≥ 8 g/dL. This method has been used in our institution for the past eight years with great success [13]. The same graphic presentation can be used to show the percentages of RBC orders that were placed for 1 unit or ≥ 2 unit units of RBCs. Given that recent guidelines have emphasised a restrictive transfusion strategy and single-unit RBC transfusions in non-bleeding,

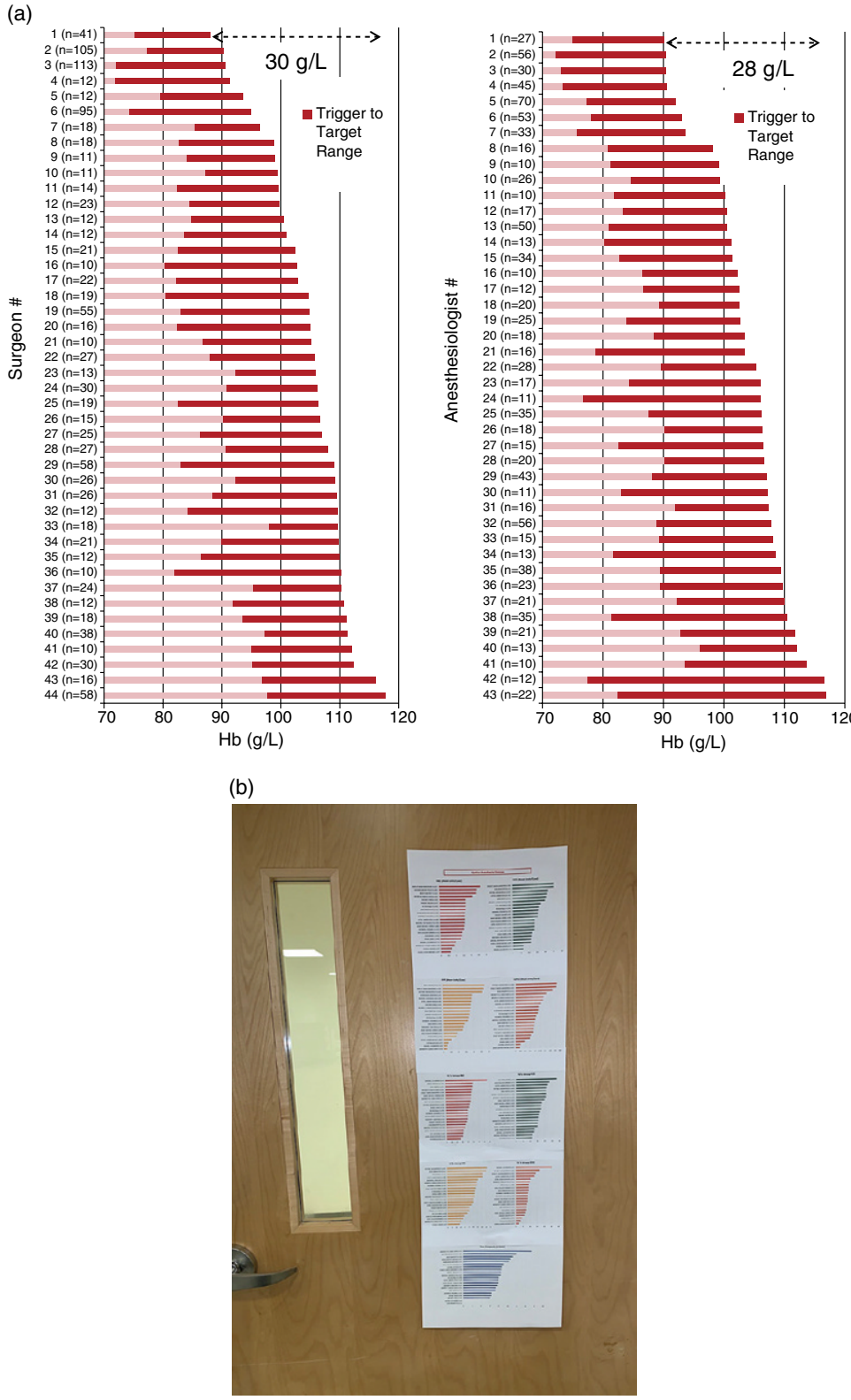

Figure 40.3 (a) Intraoperative transfusion data comparing cardiac anaesthesiologists to their peers using rank-order bar graphs showing percentage of patients transfused and average number of blood units per patient. All four major blood components are shown (red blood cells, plasma, platelets and cryoprecipitate) and the findings were displayed on the anaesthesia office door (b). This type of audit and feedback, with physicians' names on the graphs, resulted in great interest among the group and a subsequent improvement in overall blood utilisation. *Source*: Hensley et al. 2019 [12]. Reproduced with permission of John Wiley & Sons.

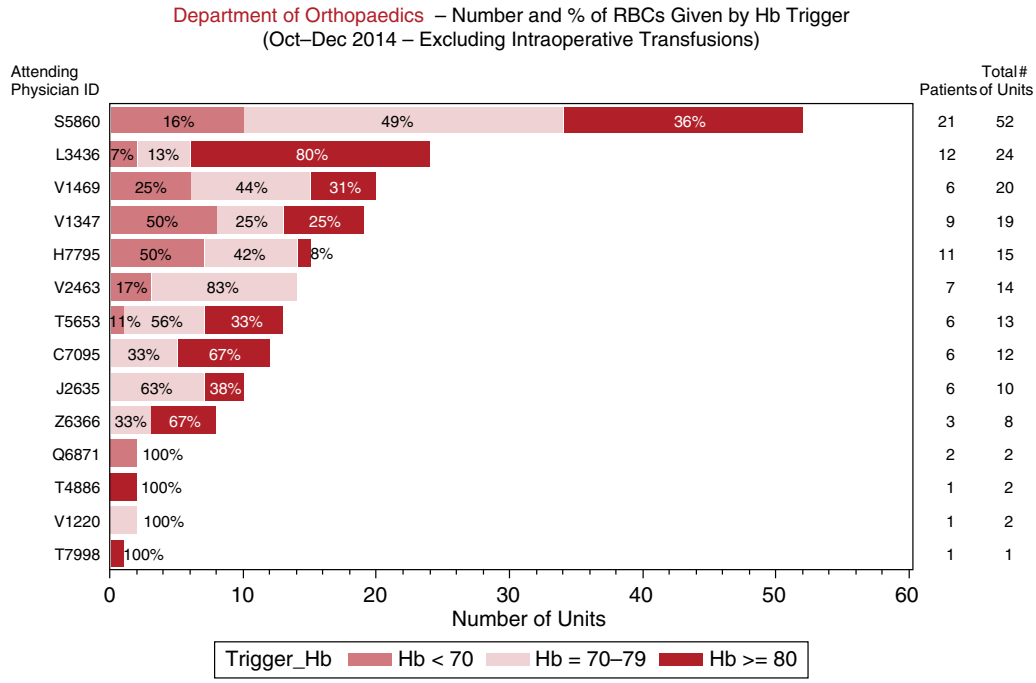

Figure 40.4 These data were taken from the computerised provider order entry system. Individual physicians (y-axis) were compared by the number of red blood cell (RBC) orders placed (x-axis) over a three-month time period, along with the percentage of orders placed by haemoglobin (Hb) trigger. The proportion of orders for which the most recent Hb preceding the order was <7 g/dL is shown in green, 7–7.9 g/dL in yellow and ≥ 8 g/dL in red. An accompanying table for each department is provided; the 5-digit codes on the y-axis correspond to the names of attending physicians.

hemodynamically stable patients [14], this rank-order bar graph method with evidence-based, colour-coded depiction of guideline compliance is easy to interpret and can have a large impact in improving practice by encouraging guideline compliance. Supporting a single-unit transfusion approach entails a careful and detailed assessment of the patient after each unit transfused. The patient should have a repeat Hb check, along with assessment of other physiological parameters such as heart rate, blood pressure and oxygen saturations, to assess clinical response or relief from prior symptoms of anaemia and prevent unnecessary subsequent transfusions.

The above-mentioned approaches assess local, departmental or hospital-wide patient data. For a broader, more comprehensive view, or to compare clinical practice between specialties, national databases, such as the Emergency Care Data Set (ECDS) or Critical Care Minimum Data Set, will become highly useful sources. These are examples available in the UK, for which applications can be made requesting the required data to NHS Digital, along with the reasons for the data. This process requires additional steps including contracts, data protection agreements and approval of ethics, where sensitive data is required. Similar databases exist in other countries that employ EMR systems to record clinical treatment, such as the American College of Surgeons National Surgical Quality Improvement Program database, or the National Patient Register in Sweden.

Clinical Outcome Data

Nothing matters more than clinical outcomes, but these can be hard to capture. Important outcomes to assess in a PBM

programme are listed in Table 40.2. Morbid events that are commonly assessed in PBM programmes may include, for example, 21 events that can be grouped into six major categories to ease interpretation and presentation of outcome data. If further simplification is needed, one can use a composite morbid event rate, which is a dichotomous outcome that includes the occurrence of any one of the individual morbid events.

Table 40.2 Clinical outcomes to assess in a patient blood management programme.

Clinical outcome	Description
Morbid events	
Infections	Surgical site infection Drug-resistant infections Sepsis *Clostridium difficile* infection
Thrombotic	Venous thrombotic event Pulmonary embolus Disseminated intravascular coagulation
Ischaemic	Myocardial infarction Transient ischaemic attack Cerebral vascular accident
Respiratory	Ventilator-associated pneumonia Acute respiratory distress syndrome (ARDS)
Renal	Renal insufficiency Renal failure – requiring renal replacement therapy
Transfusion-related morbidity	Transfusion-related acute lung injury (TRALI) Transfusion-associated cardiac overload (TACO) Allergic reactions Haemolytic reactions Febrile non-haemolytic reactions Bacterial contamination Hyperkalaemia
Mortality	In-hospital, 30-day, 90-day or longer
Length of stay	Duration of hospital stay (days)
30-day readmission rate	Readmission to the same or other hospital within 30 days post discharge

In-hospital mortality is an important clinical outcome and, unlike 30-day or 1-year mortality, is obtainable from the EMR. Length of hospital stay and 30-day readmission rate are also important outcomes to capture. It is remarkable that compared to non-transfused patients, transfused patients have a threefold higher composite morbid event rate, a threefold higher length of stay and a ninefold higher in-hospital mortality rate (unpublished data from Johns Hopkins, 2009–15). Although morbidity and mortality are clearly *associated* with transfusion, it quickly becomes apparent that transfused patients have more co-morbidities and undergo more complex procedures. This phenomenon has been referred to as 'confounding by indication'. Thus, it becomes important to risk-adjust transfusion data.

Clinical outcome data may be collected and analysed from international classification of disease (ICD-10) codes, which are determined by professional medical coders after patient discharge and entered into billing databases. Hospital-acquired morbid events of each type can be found by searching for a particular set of ICD-10 codes in the discharge data. Other sources of data that are prospectively collected include registry data, when hospitals participate in these programmes. The National Surgical Quality Improvement Program (NSQIP) and the Society for Thoracic Surgery (STS) registries are examples that include outcome data. These registries take steps to ensure completeness and accuracy of data, whereas billing databases follow complicated rules that reflect reimbursement and regulatory obligations.

Risk Adjustment

Risk adjustment is critical when considering PBM data. Sicker patients and those who undergo more invasive procedures receive more transfusions, and they are also at risk

for worse outcomes, hence the need to adjust any such outcomes with the patient-specific risk variables. In the 1980s, early attempts at risk adjustment included the Charlson Comorbidity Index [15]. This weighted score for patient co-morbidities was shown to predict mortality over both a 1-year and a 10-year period, and has also been shown to correlate with transfusion requirements.

A more recent method of risk adjustment is the case-mix index (CMI). Created by 3M (Oakdale, MN, USA), this diagnosis-related group (DRG)-based scoring system has evolved over the past two decades into the CMI values currently used to determine Medicare reimbursement – the All Patient Refined (APR)-DRG weighted score and the Medicare Severity (MS)-DRG weighted score. These scores take into account both severity of illness and complexity of procedure, ultimately by using ICD-10 codes and patient characteristics. Our group has shown a strong relationship between these CMI numbers and utilisation of RBCs, plasma and platelets (Figure 40.5) [8]. We have also shown that even after risk adjustment for CMI, implementing a PBM programme resulted in decreased blood utilisation, with either no change or improved clinical outcomes [16,17]. Thus, it is helpful to track CMI changes over time as a PBM data variable. The CMI also helps with benchmarking one institution's blood utilisation data against that of others, as this type of risk adjustment can account for differences in blood use related to case mix alone.

Crossmatch to Transfusion Ratio

Since the concept of a Maximum Surgical Blood Order Schedule (MSBOS) was first described in the mid-1970s, efforts have been made to optimise the process of ordering blood before surgery. The original MSBOS was created using data from 300 hospitals to determine the ideal blood orders for 50 common surgical procedures. Using electronic anaesthesia records that accurately record transfusion data, we have described methods for creating an institution-specific MSBOS with 135 unique categories of surgical procedures [18]. We have since shown that updating the MSBOS in this fashion can reduce unnecessary preoperative blood ordering and result in substantial cost savings, especially for cases that are rarely or never transfused [19]. Ideally, the methods we describe will allow other facilities to create their own institution-specific MSBOS using data from their anaesthesia information management systems.

Preoperative Anaemia Screening and Management

Various performance measures have been proposed to diagnose and treat preoperative anaemia before elective surgical procedures. For example, the Joint Commission (the hospital-accrediting body in the USA) has proposed, but not yet mandated, that patients who are scheduled for elective surgery and have a high probability of requiring a blood transfusion have their Hb level tested between 45 and 14 days before the procedure. The National Institute for Health and Care Excellence (NICE), the nationwide body responsible for developing evidence-based clinical policy and practice guidelines in the UK, recommends that all patients undergoing major surgery should be assessed with a full blood count a minimum of 2 weeks prior to their planned date of surgery. NICE also recommends that those found to be anaemic should be counselled regarding their risks and where appropriate have their surgery postponed until appropriate investigation and management have been completed.

The data typically collected to satisfy such performance measures include a numerator

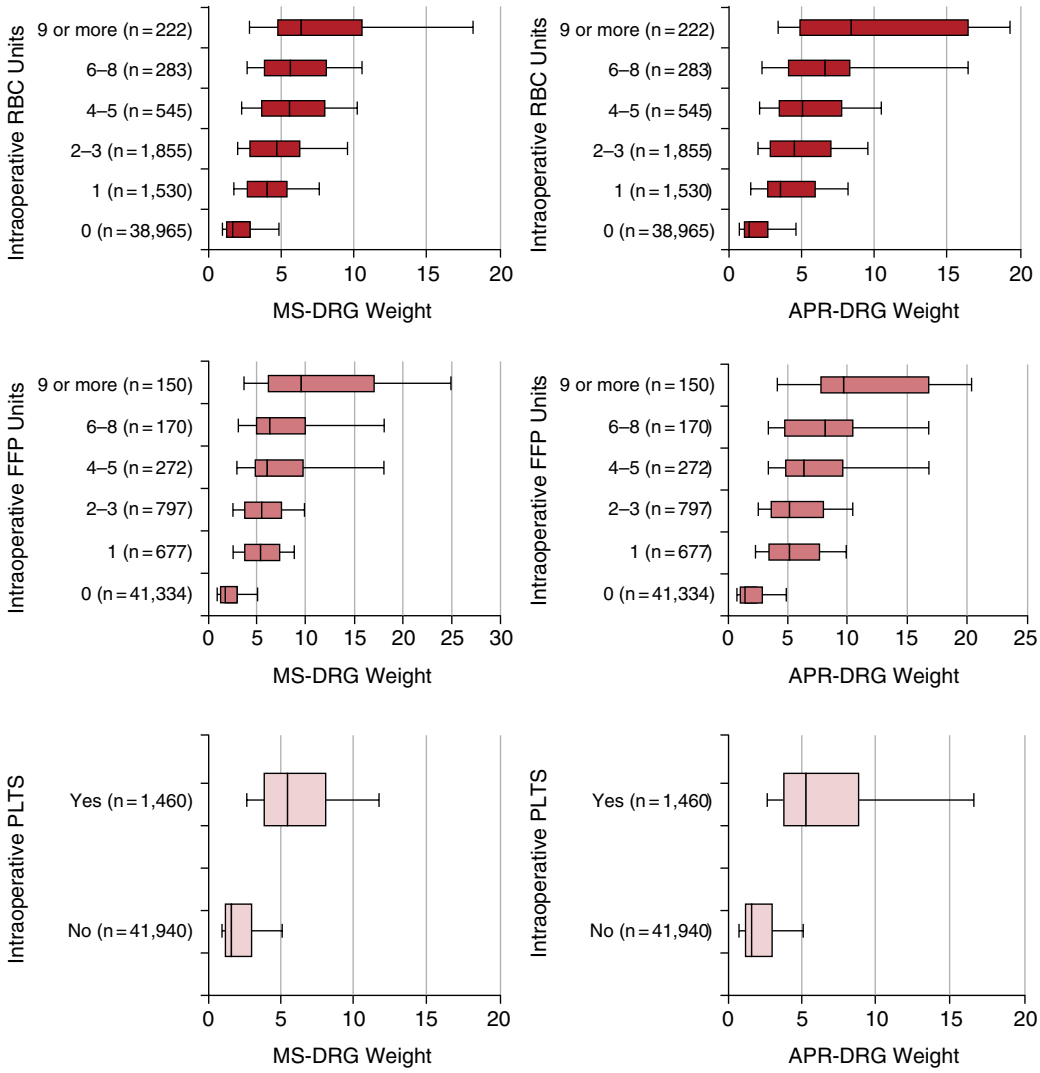

Figure 40.5 Intraoperative blood component requirements are plotted as a function of the case-mix index. Left side: the case-mix index is represented by the weighted Medicare Severity Diagnosis-Related Group (MS-DRG Weight); right side: the case-mix index is represented by the weighted All Patient Refined Diagnosis-Related Group (APR-DRG Weight). The data show a clear relationship between a higher case-mix index value and greater intraoperative transfusion requirements for red blood cells (RBCs), fresh frozen plasma (FFP) and platelets (PLTS). Differences in case-mix index values among transfusion requirement groups are significant for all six analyses shown ($p < 0.0001$). *Source*: Modified from Stonemetz et al. 2014 [8].

(number of patients with anaemia screening) and a denominator (number of patients scheduled for surgery). Recently such a measure has been proposed, along with other PBM performance measures, with the aim of collecting such data from EHRs. Because of the electronic data source for these performance measures, they have been termed 'electronic PBM (ePBM) performance measures'.

Conclusions

The variety and volume of data that can be collected to support a PBM programme are almost limitless. The challenge is to extract these data and focus on variables that will have the greatest ability to promote evidence-based practice. The methods of presenting data to providers are critical. Because blood transfusion is the most common billable procedure performed in US hospitals [20], and has also been named one of the five most overused procedures [21], managing its use has great potential to reduce risks, improve outcomes and reduce cost. Because quality / cost = value, a successful PBM programme will be an asset to any hospital or health system.

Conflicts of Interest

NBH has received consulting fees from Octapharma.

SMF has received consulting fees from Medtronic, Haemonetics and Baxter.

KEY POINTS

1) When assessing compliance with transfusion guidelines, the haemoglobin (Hb) target (after the transfusion) is equally important to the Hb trigger (prior to transfusion). The Hb target is usually 1–1.5 g/dL higher than the trigger in the randomised clinical trials that support a restrictive transfusion strategy.
2) The most useful blood utilisation metric to determine the impact of a patient blood management (PBM) programme is the average number of units transfused per admitted patient, as this parameter inherently adjusts for changes in caseload or hospital inpatient case volume.
3) Comparing providers to peers within their specialty service for Hb transfusion triggers and targets is an effective method of presenting data to improve practice.
4) Clinical outcomes that should be assessed in a PBM programme include morbid events, mortality, length of stay and 3-day readmission rate. Morbid events can be obtained from ICD-10 codes in the hospital's billing database.
5) Variables used to risk-adjust blood utilisation data include the Charlson Comorbidity Index and the case-mix index, which adjusts for both complexity of procedure and severity of illness.

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41 Regulation and Accreditation in Cellular Therapy

Julia Wolf[1], *Zbigniew (Ziggy) M. Szczepiorkowski*[2] *and James Griffin*[3]

[1] *NHS Blood and Transplant, North Bristol Park, Filton, Bristol, UK*
[2] *Transfusion Medicine Service, Cellular Therapy Center, Dartmouth-Hitchcock Medical Center and Geisel School of Medicine at Dartmouth, Hanover, NH, USA; Institute of Hematology and Transfusion Medicine, Warsaw, Poland*
[3] *Therapeutics at NHS Blood and Transplant, Filton, Bristol, UK*

In recent years there have been considerable advances in cellular therapies. Since its inception in 1968, haematopoietic stem cell transplantation (HSCT) has been the most widely used type of cellular therapy, providing a potentially curative treatment option for many haematological and non-haematological diseases. More recently advances in the use of cellular therapy for tissue regeneration and cancer immunology have led to novel therapy options in malignancy. Immune effector cell therapy (chimeric antigen receptor [CAR]-T cell) has seen particularly dramatic increases over the last five years, making an understanding of regulation and accreditation in cellular therapy essential.

Several agencies and professional bodies are involved in these processes in the USA and Europe. The regulations and standards depend on the source of the cell to be transplanted, the way it is used and the nature of any manipulations carried out. As a result, the last decades have seen a seemingly bewildering growth in regulatory and accreditation requirements, which have put pressure on both clinical and laboratory services. These are driven by a need for the following:

- Traceability of products from donor to recipient.
- Microbiological safety (related to donor/patient as well as collection/processing).
- Enhanced product quality.
- Consistent measures of product function.

Multiple organisations are involved in the process of accreditation and standard setting for HSCT programmes. Figure 41.1 illustrates the timeline of this involvement by different organisations.

Haematopoietic Stem Cell Transplant Activity

HSCTs have increased considerably in number over the last decade. While the majority are autologous, allogeneic HSCTs have increased from 3160 in 1997 to over 21 000 in 2020 [1]. The overall number of unrelated

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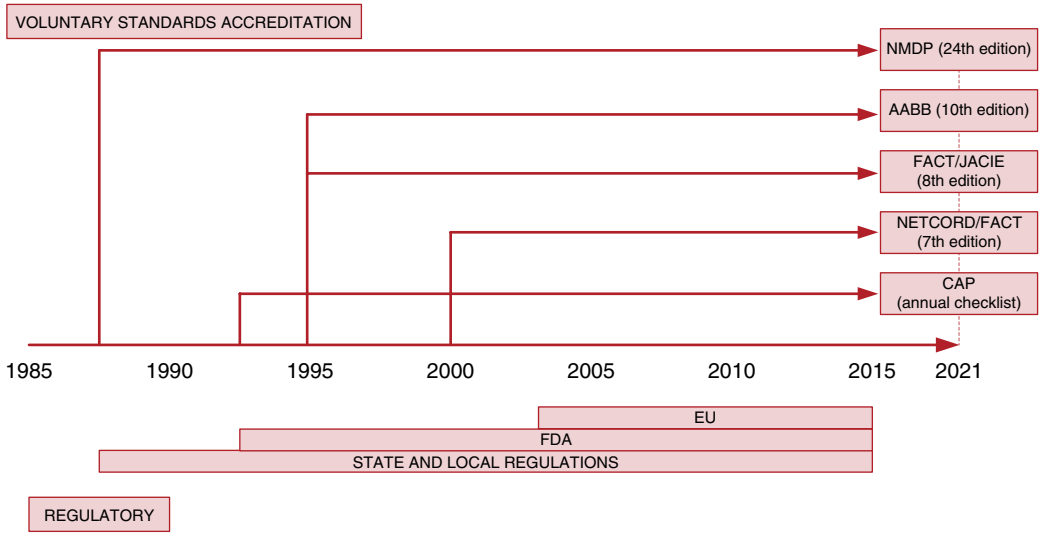

Figure 41.1 Timeline of involvement of different organisations in the field of cellular therapy. AABB (recently renamed as Association for the Advancement of Blood and Biotherapies); CAP, College of American Pathologists; FACT, Foundation for the Accreditation of Cellular Therapy; EU, European Union; FDA, US Food and Drug Administration; JACIE, Joint Accreditation Committee (International Society for Cellular Therapy [ISCT] and European Blood and Marrow Transplant Group [EBMT]); NMDP, National Marrow Donor Program.

donors available on international registries has also risen, from 4.8 million in 1997 to 39 million in 2021 [2].

Over the last two decades, there has been a switch from use of bone marrow to apheresis haematopoietic progenitor cells (HPC, apheresis – HPC(A)), which are now regarded as the source of choice in 98% of autografts and 79% of allografts in Europe and in 86% of HSCTs overall in the USA. While the use of cord blood (CB) units rose considerably in the early 2000s, the development of improved graft-versus-host-disease prevention strategies has led to preferential utilisation of cells from haploidentical donors as an alternative donor source in many centres.

In Europe, 4200 HSCTs were reported to the European Blood and Marrow Transplant Group (EBMT) in 1990, a number that rose to 48 512 in 2019. Of these, 41% were allogeneic (n = 19 798) and 59% autologous (n = 28 714) [3]; 30% of allografts are now from HLA-identical siblings and 51% from unrelated donors. Utilisation of haploidentical transplants increased from 802 in 2010 to 3538 in 2019.

HSCT has increased in all diseases reported to the EBMT and Center for International Blood and Marrow Transplant Research (CIBMTR), except for chronic myeloid leukaemia (CML), where the advent of tyrosine kinase inhibitors has reduced numbers (see Chapter 44).

CAR-T cell therapy has increased dramatically, from 151 performed in Europe in 2017 to 1134 in 2019 [3].

The Structure of Stem Cell Transplant Programmes

Figure 41.2 shows the journey of an allogeneic stem cell product from human leucocyte antigen (HLA) typing of a blood sample from a registry or sibling donor or CB unit in the histocompatibility and immunogenetics (H&I) laboratory, via the collection facility and the cell processing laboratory to the clinical transplant unit. The many relevant accreditation and regulatory bodies and their areas of involvement in HSCT are shown.

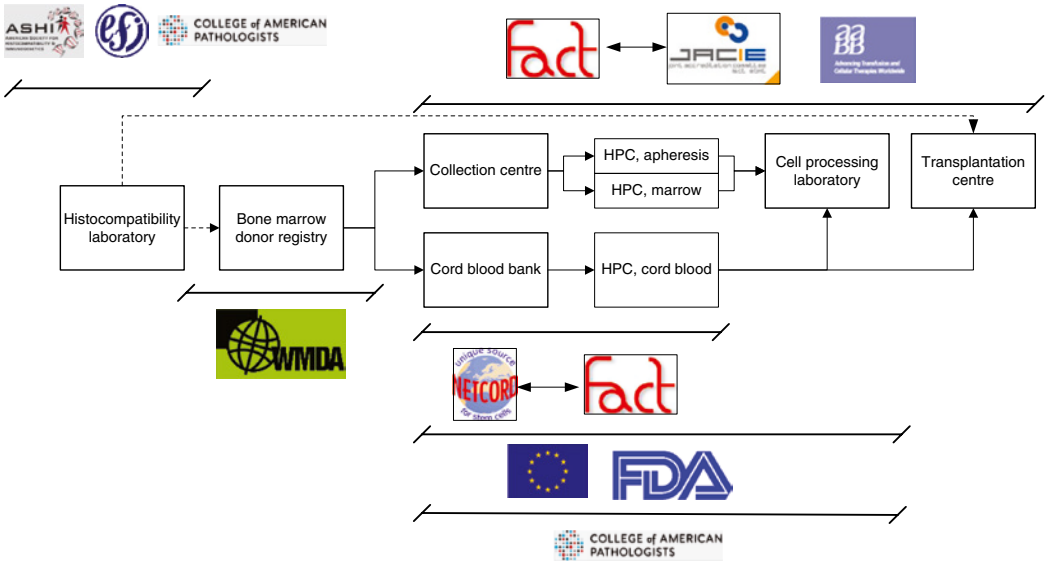

Figure 41.2 Regulatory environment for haematopoietic stem cell transplantation including immunoeffector cells. Note that this figure does not reflect all potential regulatory reporting requirements for transplantation centres including outcome reporting.

European Union Directives and Legislation

In 2004, the European Union (EU) Directive on Tissues and Cells, which defines the safety and quality standards for tissues and cells, was published as Directive 2004/23/EC [4]. In 2006, two annexes supplying more detailed technical information were published as Commission Directives 2006/17/EC (donation, procurement and testing) and 2006/86/EC (traceability requirements, notification of serious adverse events, coding, processing, preservation, storage and distribution) [5,6]. The Directives are legally binding, with a requirement that they are transposed into European law.

In 2005, national Competent Authorities (CAs), responsible for the implementation of EU legislation and Directives, were established in European Member States. In the UK, the Human Tissue (Quality and Safety for Human Application) Regulations, which translated the EU Directive into UK law, were published in 2006 and 2007; the Directive and both annexes were fully implemented. Of relevance to HSCT and immunotherapy, the following cells and tissues are included within the scope of the Directive:

- Haematopoietic stem cells from peripheral blood, bone marrow and cord blood.
- Donor leucocytes and other cellular therapies.
- Adult and embryonic stem cells.

The various sections of the Directive describe:

- The requirements for the person in charge of a cellular therapy or tissue facility (the responsible person or designated individual).
- The arrangements for the facility itself and its staffing.
- The role of the CA and the need for twice-yearly inspections.
- The requirements for consent.
- Traceability with retention of key records for a period of 30 years.
- The reporting of adverse events and reactions to the CA.
- Conditions to be met when stem cells are imported or exported.

International Identifiers: The Single European Code

Increasing use of medical products of human origin such as blood, cells, tissue, milk, and organ products in the early 1990s led to the development of a global standard for terminology, identification, coding and labelling. This standard, called ISBT 128, supports accurate and safe transfer and traceability of these products. Traceability of tissues and cells intended for human application is also a key principle of the European Directive 2006/86/EU.

In 2015, the Single European Code (SEC) was introduced to facilitate identification of all tissue and cell products previously labelled with the ISBT 128. While this became mandatory across the EU in 2017, implementation has varied between member states, with some not enforcing usage of SEC at the time of writing. The SEC consists of 1) a donation identification sequence (indicating the origin of cells) and 2) a product identification sequence (classifying the type of cells).

Certain specific situations, such as reproductive cell donation from a partner, are exempt from requiring a SEC. Systems such as SEC formed the basis for the development of GRID numbers.

The Human Tissue Act 2004

This key piece of legislation serves as a good example of how national legislation for cell and tissue collection and processing operates [7,8]. It was introduced in the UK in 2006, repealing and replacing the Human Tissue Act (1961) as well as the Anatomy Act (1989) and Human Organ Transplants Act (1989). It established the Human Tissue

Authority (HTA) as the CA for the UK. Its aim is to regulate the removal, storage, use and disposal of human bodies, body parts, organs and tissues.

One of the most important principles of the Human Tissue Act is consent. Legislation on consent varies according to whether the Act is applied to tissues from the living or the deceased. Consent is required when tissue is removed from the living or deceased for the purposes of:

- Anatomical examination.
- Determining the cause of death.
- Obtaining scientific or medical information about a person relevant to another.
- Public display.
- Research in connection with disorders or functioning of the human body (unless the material is made anonymous and for specific, ethically approved research).
- Transplantation.

The Human Tissue Act is supported by two governmental regulations – Statutory Instruments 2006 no. 1659 (37) and 2006 no. 1260 (38) – that are directions issued by the HTA to help explain and interpret the Act, as well as a number of Codes of Practice, of which three are particularly relevant to cell and tissue therapies [9]:

- Code 1: Consent.
- Code 5: Disposal of human tissue.
- Code 6: Donation of allogeneic bone marrow and peripheral blood stem cells for transplantation.

Obtaining legally valid consent is extremely important. The Human Tissue Act states that consent is a positive, voluntary act, which may be withdrawn at any time (other than where a tissue has already been used). Appropriate information should be provided and the person giving consent must have the capacity to do so.

Children may assent or consent if they are competent to do so. Even when children are not deemed competent, processes must be in place to ensure that the views of the child are considered. This includes involvement of independent assessors, who facilitate decision making on whether children can donate cells.

Consent prior to death is sufficient for organ and tissue donation and relatives have no legal right to overrule such consent. From December 2015, the Human Transplantation (Wales) Act 2013 allows the use of deemed consent for deceased organ and tissue donation in Wales; where no record of a person's decision on organ donation is held, they will be deemed to have given their consent, with some notable exemptions as detailed in the Code of Practice on the Human Transplantation (Wales) Act [10].

The Human Tissue Act also defines the responsibilities of a 'designated individual' who holds the primary legal responsibility to ensure that the requirements of the Act are complied with when procuring any tissue or cells for human application. Centres are inspected by regulatory bodies on an alternate-year basis to ensure the principles of the Human Tissue Act are followed.

International Identifiers: GRID Numbers

International identification systems such as ISBT-128 or the SEC have been used to standardise identification and labelling of cells and tissues for human application since the early 1990s. Increasing transplant activity and a growing donor pool, with over 39 million potential donors now registered worldwide, have since created a need for an improved global identification and traceability system to facilitate international collaboration.

The GRID (Global Registration Identifier of Donors) system was developed to this effect in 2019 by the World Marrow Donor Association (WMDA) in collaboration with the International Council for Commonality in Blood Banking Automation (ICCBBA) and is now mandatory for all registered centres. It reduces risks associated with misidentification of donors and provides a

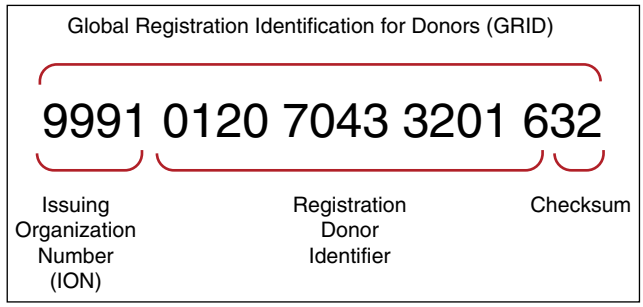

Figure 41.3 The GRID number consist of a 19-character identifier that contains an issuing organisation number (ION); a unique registration donor identifier; and a checksum – a number calculated by an algorithm that ensures the previous numbers are correct.

standard format for identifiers than can be easily read by computers and human eye (Figure 41.3) [11].

US Food and Drug Administration

The Food and Drug Administration (FDA) of the Department of Health and Human Services has been involved in cellular therapy since the early 1990s [12]. The FDA recognised the need for regulatory oversight in cell, gene and tissue therapies and products. Initial guidance documents were issued based on the Public Health Service Act, Section 361 (42 USC 264).

FDA Good Tissue Practice (GTP) Regulations for human cells, tissues and tissue-based products (HCT/Ps) require institutions shipping HPC, Cord Blood (HPC(CB)), HPC(A) and therapeutic cell, Apheresis (TC(A)), but not HPC, Marrow (HPC(M)), to be registered with the FDA as manufacturers. There are specific requirements for donors who may be eligible/ineligible based on a suitability determination and defined in final guidance documents (see below). An annual renewal is required. All FDA-registered facilities are listed on the FDA website.

The regulatory approach implemented by the FDA based on the 1997 proposal for regulation included cellular therapy products (HCT/Ps) with gene therapy and tissues rather than with blood components. This different approach had significant implications for the field by defining minimal requirements for establishments involved in manufacturing of HCT/Ps.

The FDA also introduced a concept of risk assessment that includes (1) the relationship between the donor and the recipient (i.e. autologous, allogeneic related, allogeneic unrelated); (2) the amount of processing and manipulation (non-manipulated, minimally manipulated and more than minimally manipulated); and (3) the purpose for which the tissues are used (homologous and non-homologous use, where homologous use is defined as repair, replacement or supplementation of a recipient's cells or tissues with an HCT/P that performs the same basic functions in the recipient as in the donor).

The last aspect of risk assessment has been debated by the cellular therapy community as one that potentially assigns a different level of regulatory scrutiny based on the intended use despite equivalent risk in the first two areas, the donor and the level of manipulation.

For very practical reasons, it is common among cellular therapy practitioners in the USA to discuss products as '351' and '361' products. This nomenclature relates to two different sections of the Public Health Service Act. The 361 products are covered in 21 Code of Federal Regulations (CFR) 1271 A, B, C, D, E, F (i.e. GTP), while the 351 products are covered in multiple regulations, including 21 CFR 1271 C, D; 21 CFR 207.20 (f); 21 CFR 210–211; 21 CFR 807.20 (d); 21 CFR 820.1 (a); 21 CFR 312 (investigational new drug regulations, IND) and others. The 361 products are defined as (1) minimally

manipulated; (2) intended for homologous use; (3) not involving combination with a drug or a device, except for a sterilising, preserving or storage agent, if the agent does not raise new clinical safety concerns; and (4) not having a systemic effect and not dependent upon the metabolic activity of living cells for its primary function, or having a systemic effect and being for autologous use, or for allogeneic use in a first-degree and second-degree blood relative or for reproductive use. All products that do not fulfil these requirements are considered 351 products.

Based on the assignment of 351 and 361 products, there are different requirements for biological product deviation reporting.

It is important to note that there are tissues excluded from 21 CFR 1271, which include vascularised organs, whole blood and blood components, human milk and minimally manipulated bone marrow. Thus, HPC(M) (minimally manipulated) is regulated by a different set of regulations, which are under the authority of the Health Resources and Services Administration (HRSA).

For a thorough discussion of FDA regulatory activities and current guidance documents, the reader is referred to the agency website: www.fda.gov/BiologicsBloodVaccines/default.htm. The FDA has issued several new guidance documents addressing novel cellular therapy products and the regulatory pathway to approval.

The ultimate goal of the FDA regulatory structure is to bring cellular therapy products to licensed status. This is particularly apparent with new immune effector products. Currently (April 2021) there are 20 FDA-licensed cellular and gene therapy products, i.e. 8 CB products, 6 immune effector cells (regulatory T cells, T-regs; dendritic cells, DCs), 3 virally based therapies and 3 other products [13].

The example of HPC(CB) illustrates the limitations of this approach for HPCs. In October 2009, the FDA issued a guidance document regarding the biological licensed application for HPC(CB). The regulations required that after October 2011, all CB units would be either licensed by CB banks or issued based on the IND pathway. These requirements led to a significant effort by the CB banks to submit a biological licence application (BLA) to the FDA for approval. At the time of writing, eight products – Hemacord (New York Blood Center); HPC(CB) (Clinimmune Labs, University of Colorado Cord Blood Bank); Ducord (Duke University School of Medicine); Allocord (SSM Cardinal Glennon Children's Medical Center); HPC(CB) (LifeSouth Community Blood Centers); Clevecord (Cleveland Cord Blood Center); HPC(CB) (MD Anderson Cord Blood Bank); and HPC(CB) (Bloodworks) – have been licensed. Only three new CB products were licensed in the period from November 2015 to April 2021. There is growing concern that many CB banks would not be able to meet the heavy burden of BLA and the licensing process. This, paired with decreased utilisation of HPC(CB) worldwide, creates a significant risk for economic survival of CB banks in the USA.

The CB units that are not licensed are issued under IND protocol (e.g. NMDP is a holder of one of the INDs).

Non-governmental (Voluntary) Accreditation

Many programmes elect to be accredited by one of the voluntary accrediting organisations, in addition to observing governmental regulations. There are multiple reasons for voluntary accreditation, ranging from recognition by healthcare insurance providers for reimbursement purposes through to improved quality of care and fulfilling requirements of some of the local governmental regulations, e.g. the Commonwealth of Massachusetts (USA) requires Foundation for the Accreditation of Cellular Therapy (FACT) accreditation from all transplantation centres. The NHS England Clinical Commissioning Policy for Haematopoietic Stem Cell Transplantation now requires HSCT providers to be accredited by the

Joint Accreditation Committee of the ISCT (JACIE) [14].

All accrediting organisations require adherence to local and governmental laws and regulations in addition to individual standards established by each of them. The standards are prepared by a group of experts, typically called the Standards Committee, which establishes minimum expectations for the facilities willing to participate in the accrediting programme. The published standards, which are updated at defined time intervals, are then used as a tool during the inspection process.

In some countries reimbursement for transplant activity requires that individual centres are accredited by national organisations for the work carried out, e.g. JACIE accreditation in England or National Association of Testing Authorities (NATA) accreditation in Australia.

Table 41.1 summarises the major differences (and similarities) between different accrediting organisations.

The accreditation process generally consists of three phases:

- Phase I (Application): the applicant facility submits necessary documentation to the accrediting body and certifies its compliance with the standards.
- Phase II (Confirmation): the accrediting body, using on-site inspection, confirms that the applicant facility truly follows the standards.
- Phase III (Recognition): a certificate of accreditation is issued based on the documentation submitted and the results of on-site inspection and, if necessary, satisfying responses to identified shortcomings in the applicant facility.

The accreditation certificate has an expiry date and stipulates that any significant changes in the programme structure and/or performance will be promptly reported to the accrediting body. Each of the accrediting organisations may have additional requirements.

FACT and JACIE

In 1996 the Foundation for Accreditation of Cell Therapy (FACT) in the USA initiated a voluntary inspection and accreditation scheme for cell therapy facilities. Two years later, its European counterpart, the Joint Accreditation Committee of the ISCT (International Society for Cell Therapy) and EBMT (JACIE), was founded. FACT–JACIE is a voluntary system that accredits clinical transplant programmes as well as the cell collection, processing and banking elements that are covered by current EU legislation. While FACT–JACIE accreditation is not mandatory, it provides a means whereby transplant facilities can demonstrate that they are working within a quality system that covers all aspects of the transplantation process. In the UK, accreditation is required by the NHS for reimbursement of transplant or immune effector cell activity.

The primary aim of FACT–JACIE is to improve the quality of HSCT through external inspection of facilities, ensuring compliance with the FACT–JACIE standards. A further aim is to ensure consistency between these standards and other national and international standards, including the EU Tissues and Cells Directive (Directive 2004/23/EC) and the related Commission Directives 2006/17/EC and 2006/86/EC (see above).

FACT–JACIE Standards

Initial standards published by FACT–JACIE covered patient care during the transplant period; donor care during stem cell collection; and laboratory processing, storage and delivery of HPCs. With rapid advancement in stem cell research and therapy, which now also includes other cellular, regenerative and immune therapies, standards have been regularly updated. Current 2021 standards apply to the use of stem cells and TCs derived from the peripheral blood or bone marrow, including donor lymphocytes and mesenchymal stem cells [15]. It additionally covers the clinical use of HPC(CB), but not the collection or banking of CB, which is covered

Table 41.1 Overview of voluntary accreditation/registry organisations (see individual websites to identify accredited centres, laboratories and registries, as these data change often).

	FACT	JACIE	NetCord/FACT	AABB	CAP	NMDP	WMDA
Membership	No	No	No	Yes	Yes	Yes	Yes
Accreditation	Yes	Yes	Yes	Yes	Yes	No	Yes
Scope							
Registries	na	na	na	na	na	na	++
Recruitment	na	na	na	na	na	++	+
Donor	+	+	++	+	+	++	++
Collection	++	++	++	++	++	++	+
Processing	++	++	++	++	++	++	na
Transplantation	++	++	na	+	na	++	na
Products							
HPC, Apheresis	Yes	Yes	No	Yes	Yes	Yes	Yes
HPC, Marrow	Yes	Yes	No	Yes	Yes	Yes	Yes
HPC, Cord Blood	No	No	Yes	Yes	Yes	Yes	Yes
TC, Lymphocytes	Yes	Yes	No	Yes	Yes	Yes	No
Other CTCs	Yes	Yes	No	Yes	No	No	No
Standards structure	Checklist	Checklist	Checklist	ISO based	Checklist	Checklist	Checklist
Current edition/version	8th	8th	7th (2020)	10th	2022	24th	2020

AABB, Association for the Advancement of Blood and Biotherapies; CAP, College of American Pathologists; CTC, cellular therapy products; FACT, Foundation for the Accreditation of Cellular Therapy; HPC, haematopoietic progenitor cells; ISO, International Organization for Standardization; JACIE, Joint Accreditation Committee (International Society for Cellular Therapy [ISCT] and European Blood and Marrow Transplant Group [EBMT]); na, not applicable; NMDP, National Marrow Donor Program; TC, therapeutical cell; WMDA, World Marrow Donor Association.

by the related NetCord–FACT standards and inspected and accredited by NetCord–FACT [16]. The eighth-edition standards were published in 2021, with expectation for review on a bi-annual basis (reviews are currently three yearly but will increase in frequency with the latest publication of standards). An up-to-date list of standards can be found at https://www.ebmt.org/jacie-accreditation.

Recent progress in the field of CAR-T therapy has resulted in new challenges in accreditation. Existing processes had to be adapted for this novel technology and are complicated further by requirements relating to licensing for pharmaceutical companies. This has resulted in the development of education programmes, audit processes and data management requirements.

Quality and Quality Management

An active quality management programme (QMP) is essential to the FACT–JACIE standards. A QMP ensures that all staff members carry out procedures in line with agreed standards and that these standards of practice are maintained. In a transplant programme, this facilitates collaborative working between clinical, collection and laboratory units to achieve good communication, effective common work practices and increased safety for patients. QMPs are a means of rapidly identifying errors and resolving them to minimise the possibility of repetition. They assist in training and clearly identify the roles and responsibilities of all staff. QMPs are dynamic systems that evolve with activity and ensure an ongoing commitment to quality and safety.

Within cellular therapy, QMPs relating to microbiology, H&I and immunophenotyping laboratories are particularly important. Quality managers are responsible for documenting training and development, personnel and staffing, equipment, internal and external quality control, audit, standard operating procedures and annual management review. They must also ensure that their laboratory works in accordance with and is accredited by internationally agreed standards (ISO).

The culture and systems for quality management are well established in laboratories but are relatively new in clinical units. It is recommended that HSCT programmes have dedicated quality managers.

In the UK, the United Kingdom Accreditation Service (UKAS) acts as the national accreditation body. It is recognised by the government and works to assess haematology and stem cell laboratories against the internationally agreed standard ISO15189. Similar accreditation bodies exist internationally. It should be noted that UKAS accreditation will continue to be recognised by the EU even after the UK leaves the EU.

Good Manufacturing Practice

Good manufacturing practice (GMP) describes the minimum standard that must be met by anyone producing medicinal products [17]. This includes the manufacture of advanced therapy medicinal products (ATMPs), such as cellular therapies for human application.

Data and Data Management

Activities of institutions working with human tissues, cellular therapies and ATMPs generate vast amounts of data. To ensure adherence to the regulatory and accreditation standards set out above, it is essential that all programmes record and retain these data systematically.

Data must be up to date, collected in an organised fashion, stored securely and utilised appropriately to ensure that a programme complies with local, national and international standards.

Experience of Centres Implementing FACT and JACIE

It was anticipated that implementation of the FACT–JACIE standards would pose some difficulties for applicant centres, particularly in relation to establishing a quality management system (QMS). To assess this in Europe, a survey of centres preparing for JACIE

accreditation was designed. This demonstrated that the implementation of a QMS, design of adverse event–reporting systems and production of other documentation were the most difficult aspects of preparation. All centres felt that accreditation was worth the effort invested [18]. In addition, with the implementation of the EU Directive on Safety of Tissues and Cells (2004/23/EC), it is likely that collection and processing facilities will increasingly view compliance with JACIE standards as important in providing evidence that they are complying with the requirements of the Directive.

NetCord–FACT

In the same way that FACT and JACIE cooperate to produce a globally agreed set of standards and a guidance manual for accreditation of HSCT programmes, FACT collaborates with NetCord, which is an international organisation for CB banking. Their combined international standards, first issued in 2000, are the gold standard for CB banks worldwide. The most recent standards (seventh edition) were published in 2020 [16].

AABB

Established in 1947, the AABB (recently renamed as Association for the Advancement of Blood and Biotherapies) is an international, not-for-profit association dedicated to the advancement of science and the practice of transfusion medicine and related biological therapies. The AABB approach to the field of cellular therapies has aimed to balance flexibility in an outcome-based approach with the need for rigorous evidence-based standards. The standards are written using an ISO-based template. The 10 chapter headings are based on the AABB Quality System Essentials (QSEs), published in 1997 as AABB Association Bulletin 97-4. The 10 QSEs correlate directly with ISO. The AABB Standards for Cellular Therapy Services (10th edition published in 2021 [19]), which are revised and updated every 24 months, cover all cellular therapy products and cell sources, including autologous, allogeneic and cadaveric donors, including, for the first time, clinical services. AABB also coordinates the production of a cell therapy product Circular of Information (COI) [20].

Under a QMS approach, each chapter progresses from general policies to specific procedures. The chapters are:

- Organisation
- Resources
- Equipment
- Agreements
- Process Control
- Documents and Records
- Deviations and Nonconforming Products or Services
- Internal and External Assessments
- Process Improvement
- Safety and Facilities

AABB accreditation is valid for two years and each accredited institution is assessed every 24 months. Recently, the AABB, following other accrediting organisations, introduced unannounced assessments. These occur on any day within 90 days of the accreditation expiry date.

College of American Pathologists

The College of American Pathologists (CAP) is a medical society serving nearly 16 000 physician members and the laboratory community throughout the world [21]. It is the world's largest association composed exclusively of pathologists and is widely considered the leader in laboratory quality assurance. More than 6000 laboratories are accredited by the CAP and approximately 23 000 laboratories are enrolled in the College's proficiency testing programmes. There are two proficiency tests currently offered for cellular therapy products: Stem Cell Test and Cord Blood Test.

The CAP primarily accredits laboratories in clinical and anatomical pathology, but the accreditation process also includes other entities such as cellular therapy laboratories, HLA laboratories and reproductive laboratories.

The accreditation process, called the Laboratory Accreditation Program, is based on fulfilling the CAP checklist (self-assessment and on-site inspection), which consists of three major parts: the discipline-specific checklist(s), the laboratory general checklist and all common checklists. Each checklist component consists of subject header, declarative statement and evidence of compliance. The questions on tissue banking were added to the transfusion medicine checklist in 1993. There were five questions covering (1) the authority, responsibility and accountability of the programme; (2) processing and infectious disease testing for each tissue stored; (3) procedures defining storage conditions of the different tissues handled and retention of records; (4) records showing proper storage conditions; and (5) records allowing for identification of the donor and recipient for each tissue handled. In 2004 and more recently in 2020, the CAP expanded the tissues and the HPC sections of the checklist. In general, new editions of CAP checklists are released once a year; however, some important updates can be issued more frequently.

The CAP inspection is performed every other year and generally lasts two days. The accredited facilities are also required to perform self-evaluation in the year when there is no on-site inspection.

World Marrow Donor Association

The WMDA is an international organisation that publishes standards to which HSCT donor registries wishing to achieve accreditation for their activities must adhere [2]. These standards are available on the WMDA website (www.worldmarrow.org). Important areas described by the standards include general organisation of the donor registry, donor recruitment, assessment, counselling, histocompatibility and immunogenetic testing of donors, other testing, including an infectious disease marker, information technology requirements, donor searches, collection and transport of cells. At the present time, accreditation is given after a detailed review of documentation submitted by the registry by independent reviewers, but site visits are not done, although a pilot scheme to introduce them is underway. Accreditation is valid for five years.

Histocompatibility Accreditation

The American Society for Histocompatibility and Immunogenetics (ASHI) and its European counterpart, the European Federation of Immunogenetics (EFI), accredit H&I laboratories after reviewing documentation and conducting a site visit [22,23]. The CAP also accredits histocompatibility laboratories. The accreditation by CAP fulfils requirements of the National Marrow Donor Program 24th edition of standards (October 2018). In 2019, FACT–JACIE announced that CAP standards would be acceptable and equivalent to ASHI to fulfil standards requirement. This change was welcomed by many CAP-accredited laboratories.

Advanced Therapy Medicinal Products

ATMPs are medicines intended for human application that are produced from genes, tissues or cells. This includes immune effector cell therapies. Their production, storage, use and disposal are governed by Directive 2001/83/EC and authorised centrally by the European Medicines Agency (EMA). In 2016 the sixth edition of JACIE standards was upgraded to include immune effector cells. Subsequent editions will continue to incorporate and further develop this.

As already described, the advent of immune effector cell therapy and other advanced medicinal products has provided a challenge to the delivery of care and to the accreditation and regulation environment. A further complexity arises when considering that the resulting product is also classed as a drug, necessitating close collaboration between laboratory and production unit authorities and pharmaceutical regulators.

Conclusion: How Do Haematopoietic Stem Cell Transplantation Programmes Respond to the Challenge?

The requirements of regulatory and accreditation bodies place huge demands on transplant programmes. In some cases, programmes may need to construct new and improved facilities for haematopoietic stem cell collection, processing and storage. A key feature is the need to develop robust QMPs, as described above, which will include detailed policies and procedures to cover all their activities. Initial staff training and ensuring ongoing competency are crucial. HSCT programmes should remember that deficiencies commonly found at inspection involve the QMP, policies and procedures, donor assessment and testing and the labelling of cell therapy products. The interaction between the different component parts of programmes should work seamlessly and where, for example, cell processing or laboratory testing is performed outside the programme by external agencies, service-level agreements will need to be in place. Most units that achieve compliance with regulatory and accreditation standards feel that the exercise has been worthwhile and that the quality of the services they offer has been improved.

The COVID-19 pandemic has added additional challenges for HSCT laboratories. Many aspects of operations have been affected, including transportation as well as supply chains. Furthermore, many accrediting organisations moved to virtual inspections, which resulted in challenging conditions for HSCT laboratories. It is likely that these changes may impact future delivery of inspections and assessments, though to what extent remains unclear at this point.

KEY POINTS

1) There have been considerable advances in cellular therapy in the last 20 years and newer developments include the use of cell therapy products for regenerative medicine and immunotherapy.
2) The accreditation and regulatory environments have become increasingly complex and their aim is to enhance product quality and safety.
3) The development of robust quality systems is central to achieving compliance with these new requirements.
4) Some regulations are mandatory, e.g. the EU Directive and FDA requirements, while others, such as FACT–JACIE or AABB accreditation, are voluntary.
5) Increased resource is required to successfully implement the changes needed to achieve compliance.

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42 Stem Cell Collection and Therapeutic Apheresis

Khaled El-Ghariani[1] and Zbigniew (Ziggy) M. Szczepiorkowski[2]

[1] *NHS Blood and Transplant; Sheffield Teaching Hospitals NHS Trust, University of Sheffield, Sheffield, UK*

[2] *Transfusion Medicine Service, Cellular Therapy Center, Dartmouth-Hitchcock Medical Centre and Geisel School of Medicine at Dartmouth, Hanover, NH, USA; Institute of Hematology and Transfusion Medicine, Warsaw, Poland*

The word apheresis is derived from the Greek meaning 'a withdrawal'. Therapeutic apheresis is the process of using apheresis technology to manipulate a patient's circulatory contents by removal or reduction of a substance(s) implicated in the pathophysiology, for therapy. Plasma exchange is the process of exchanging part of the patient's plasma with suitable replacement fluid. Different cellular components can be removed with high precision. Red cells can be exchanged, circulating stem cells and lymphocytes can be collected for transplantation, and the excess white cells or platelets in myeloproliferative disorders can be removed. Low-density lipoproteins and immunoglobulins can be specifically removed through the use of adsorption columns. A decision to offer these treatments to patients should be based on the benefits of apheresis, potential adverse effects, and the availability and efficacy of other treatment modalities.

Cell Separators

Efficient cell separators are currently available and are equipped with sophisticated software and safety alarm systems to detect air and changes in access or inflow pressure [1]. Apheresis technology is based on either filtration or centrifugal systems. Filtration systems use permeable membranes to separate blood into its cellular and non-cellular components by subjecting it to sieving through a membrane with suitably sized pores, for example the HF440 (Infomed, Meinier, Switzerland). Centrifugal systems use the forces generated by rotation (quantified as multiples of the force of gravity or 'g forces') to separate blood into different components. Centrifugation of blood sediments its components into distinct layers. Based on increasing density, these layers are plasma, platelets, monocytes, lymphocytes and haematopoietic progenitor cells (HPCs), granulocytes and red cells.

Apheresis machines use either continuous- or intermittent-flow technology. In the continuous-flow machines, blood is continuously pumped into a spinning disposable harness where separation takes place, and components are either diverted to a collection bag or returned to the patient as required. These machines often require two points of access to the circulation, one for withdrawal and another for return of blood, for example the Spectra Optia® (TerumoBCT, Lakewood, CO, USA), Amicus® (Fresenius Kabi, Bad Homburg, Germany) and COM.

Practical Transfusion Medicine, Sixth Edition. Edited by Michael F. Murphy, David J. Roberts, Mark H. Yazer and Nancy M. Dunbar.

TEC® (Fresenius Kabi). Intermittent-flow machines collect blood into a bowl during the draw cycle and then centrifuge blood to separate plasma and cellular components. Different components are diverted to the collection bag or returned to the patient with replacement fluid during the return cycle by a single point of access to the circulation, for example the Therakos Cellex® (Mallinckrodt Pharmaceuticals, Bedminster, NJ, USA). Apheresis systems are primed with normal saline to displace air from the harness and to ensure isovolaemia, an important prerequisite for patients with haemodynamic instability or sickle cell disease. In children and small adults, the extracorporeal volume may be relatively high, and the system will need to be primed by a mixture of red blood cells and normal saline or albumin. Cell separators must be qualified and maintained according to the manufacturer's recommendations and must be operated by trained personnel.

Patient Assessment and Treatment Planning

A physician experienced in the use of cell separators should undertake clinical assessment, to weigh the expected benefit against potential risks and inconvenience. Plasma exchange often provides only temporary relief of the patient's symptoms and it is usually only one part of the overall treatment plan. Valid consent must be obtained from all patients and donors. Initial and repeat laboratory evaluations should be tailored to the patient's clinical status and may include a full blood count, coagulation screen and biochemistry. Apheresis treatment plans will include the type of vascular access, volume to be exchanged, type of replacement fluid, frequency of procedures and monitoring of response to therapy [2].

Adequate vascular access is crucial. Peripheral veins, usually located in the antecubital fossa, should be evaluated by apheresis staff early in planning and should be used wherever possible, especially for a limited number of procedures. Central venous catheterisation is required for patients who have inadequate peripheral veins or who require frequent procedures. A rigid double-lumen catheter should be used. Trained staff must undertake central vein cannulation and post-insertion catheter care to avoid failure of vascular access during the procedure [3].

Haematopoietic Progenitor Cell Mobilisation

Currently, haematopoietic cell transplantation in adults is more commonly undertaken using mobilised peripheral blood rather than bone marrow (see also Chapter 44). This is because HPCs derived by apheresis engraft faster than marrow-derived HPCs and are harvested without hospital admission or general anaesthesia. In the steady state, circulating HPCs comprise only 0.1% of the total white blood cell count. To ensure adequate graft, mobilisation of HPCs from the marrow into the peripheral circulation is achieved by granulocyte colony-stimulating factor (G-CSF) in healthy donors, and by G-CSF and/or the administration of chemotherapy such as cyclophosphamide or disease-specific combination chemotherapy in patients undergoing autologous HSCT [4,5].

The cellular and molecular mechanism of HPC mobilisation is complex [6]. One of the pathways involves CXCR4, expressed by HPCs among other cells, and its ligand, stromal-derived factor 1 (SDF-1; CXCL12), which is produced by marrow stromal cells. The association of CXCR4 with its ligand mediates stem cell homing, trafficking and retention. Proteolytic enzymes, such as elastase, cathepsin G and matrix metalloproteinase-g, released from neutrophils following administration of chemotherapy and/or G-CSF, are thought to degrade molecules such as CXCR4 and SDF-1, which are important for anchoring stem cells to marrow stroma and inducing mobilisation. Also, G-CSF may have an inhibitory effect on expression of CXCR4 mRNA, so enhancing mobilisation. Other pathways for mobilisation include bone marrow cells such as macrophages and osteoclasts, as well as the complement system and the sympathetic nervous system [6].

Most healthy donors are mobilised by G-CSF 10 μg/kg/day. Progenitor cells usually

peak after the fourth injection when harvesting starts, and the procedure may be repeated until the target number of stem cells is achieved. Donor age, steady-state CD34 levels and the dose of G-CSF may impact the CD34+ cell mobilisation. G-CSF is effective and reasonably safe [7]. The most common side effects of G-CSF are bone pain, headaches, fatigue and nausea, and less common but more serious effects of G-CSF include hypoxaemia and splenic rupture, either spontaneously or precipitated by minor trauma or viral infection. Donors are encouraged to report any pain or discomfort that they may experience over the splenic region. G-CSF has a procoagulant effect and may increase the risk of myocardial infarction or ischaemic strokes in susceptible individuals. Current data do not suggest an association of G-CSF with genomic instability or leucaemogenesis [8]. Moreover, donor registries have identified a few cases of intracerebral bleeding among allogeneic unrelated donors. These complications may have originated from previous history of concussion or head injuries and donors with such history may not be allowed to receive G-CSF.

Two branded forms of G-CSF (Granocyte®, Chugai Pharmaceutical, Tokyo, Japan; and Neupogen®, Amgen, Thousand Oaks, CA, USA) have been available since the early 1990s. Extensive data are available concerning their safety. Recently, G-CSF biosimilar agents have become available. Reviews have shown no difference between biosimilar and reference products in biological mode of action or side effects, and they are now used routinely to mobilise autologous stem donations [9]. Recently the World Marrow Donor Association (WMDA) recommended that filgrastim biosimilar can be used for the mobilisation of stem cells in healthy donors [10].

Autologous stem cell transplantation is regularly used to restore haematopoiesis after high-dose therapy, mostly for patients with myeloma and relapsed lymphoma. Grafts for transplantation can be collected following G-CSF monotherapy at a dose of 10 µg/kg/day for 5–7 days, followed by apheresis collection of cells [4,5]. More frequently, chemotherapy is added to G-CSF during mobilisation to provide further anti-tumour effects and to increase the probability of collecting higher numbers of CD34 cells. However, this makes the cell mobilisation window less predictable and may increase treatment toxicity and related morbidity [4,5], and it is preferable to collect stem cells following a course of chemotherapy used primarily to treat or salvage the patient. The choice of chemotherapeutic agents depends on the disease characteristics and local guidelines, and should take into consideration new and developing therapeutic agents [5].

Poor Mobilisation: The Use of Plerixafor

The response of individuals to mobilisation regimens is variable and failed mobilisation of HPCs can be practically defined as a peripheral blood (PB) CD34+ cell count of less than 20 cells/µL. The mechanism of poor mobilisation in healthy donors is unclear, but may have a genetic basis. Bone marrow harvesting is mainly used to salvage the situation.

In the autologous setting, the average mobilisation failure rate in myeloma and lymphoma patients is 20%, but can be as high as 40% in certain patient groups. Mobilisation failure is defined as the inability to collect the minimum recommended stem cell dose for autologous transplant, which is 2×10^6 CD34+ cells/kg recipient body weight [4], with patients with counts less than 5 cells/µL considered absolute poor mobilisers [5].

Factors associated with poor mobilisation include stem cell damage due to old age, previous exposure to chemotherapy and radiotherapy, disease involvement of bone marrow, the use of stem cell–toxic agents such as melphalan, carmustine, fludarabine, alkylating agents or lenalidomide, the diagnosis of non-Hodgkin lymphoma and coexisting diabetes [4]. However, the PB CD34+ cell count just before leucocytapheresis is considered the most predictive factor for mobilisation failure [4,5].

The management of mobilisation failure has improved with better understanding of

the biology of stem cells and the availability of new therapeutic agents such as plerixafor. Box 42.1 lists the options available to predict and manage poor mobilisation.

Plerixafor (Mozobil®, Sanofi, Bridgewater, NJ, USA) is a CXCR4 antagonist that inhibits its interaction with SDF-1 and releases HPC into the circulation [6]. Plerixafor synergises with G-CSF and is usually administered the night before the planned first day of collection, at 240 μg/m^2 subcutaneous injection to provide safe and effective mobilisation of stem cells [4,5]. Although plerixafor is expensive, its judicious use in a selected patient population is cost-effective [11–13] and the drug has been approved by the US Food and Drug Administration (FDA) and the European Medicine Evaluation Agency (EMEA) for autologous HPC donations for patients with myeloma and non-Hodgkin lymphoma. Because most patients are good mobilisers, the universal use of plerixafor is not justified.

Plerixafor can be used in combination with G-CSF in patients who have previously failed mobilisation, with a success rate of up to 70% [10,12]. Use of plerixafor during the first mobilisation of selected high-risk patients could be more helpful by eliminating the need for a second mobilisation, by adopting the pre-emptive course otherwise known as a 'just-in-time' approach, where patients' blood CD34+ cell counts on the predicted day of harvest are monitored. Those with low CD34+ cell count (fewer than 10 per μL) would be identified as potential mobilisation failures and given plerixafor during their first mobilisation attempt [4,5,12]. However, many centres in the USA administer plerixafor pre-emptively in patients with a circulating CD34+ cell count between 10 and 20 cell/μL. Options to manage mobilisation failure are summarised in Box 42.1.

Box 42.1 Options to predict and manage mobilisation failure.

- Patients should be considered for stem cell mobilisation and harvesting, if required, early in the course of their treatment and before stem cell–toxic agents are used. Patients with high risk of mobilisation failure should be carefully monitored during the mobilisation process.
- Plerixafor is used pre-emptively in patients with low circulating CD34+ cells on the predicted day of harvest to prevent mobilisation failure as follows:
  - CD34+ cell count > 20 cell/μL: proceed with leukapheresis.
  - CD34+ cell count 10–20 cell/μL: consider other risk factors as well as the total CD34+ cell dose required. Patients may require plerixafor.
  - CD34+ cell count < 10 cell/μL: pre-emptive plerixafor is required to rescue the collection.
- Patients who have failed a mobilisation attempt could be rescued by further mobilisation attempts using granulocyte colony-stimulating factor and plerixafor (± chemotherapy).
- The use of large-volume apheresis, new mobilising agents (within clinical trials) and marginally low numbers of stem cells for transplantation are options that clinicians could consider on an individual basis.
- Bone marrow harvested from poor mobilisers may not be of good enough quality and delayed engraftment may follow.

Source: Adapted from the American consensus guidelines [4] and the European position statement [5].

Peripheral Blood Haematopoietic Progenitor Cell Collection (Leucocytapheresis)

Leucocytapheresis, following chemotherapy and G-CSF mobilisation, could commence when leucocyte counts are rising (≥ 1 × 10^9/L). However, most centres use CD34 expression on peripheral blood mononuclear cells (PBMCs), measured by flow cytometry, to predict the optimal time to start HPC collection, to predict the success of collection and to enumerate HPC in the collected product [4]. CD34 is a heavily

glycosylated phosphoglycoprotein expressed on progenitor cells of all lineages within the lymphohaemopoietic system, but not on mature cells, and is a surrogate marker for stem cells. Approximately 1.5% of aspirated normal marrow mononuclear cells, less than 0.1% of non-mobilised PBMCs and approximately 0.5% of cord blood cells are CD34+. Purified autologous CD34+ cells mediate haemopoietic engraftment, whereas CD34– cells do not engraft, except for early progenitor CXCR-4+ cells. The number of CD34+ cells infused is clearly correlated with both neutrophil and platelet recovery post transplant. Compared with marrow harvests, G-CSF mobilised grafts contain 3–4-fold higher CD34+ cells and a 10–20-fold increase in CD3+ T cells.

To ensure timely engraftment and graft survival, there is a consensus to infuse at least 2.0×10^6/kg recipient bodyweight of CD34+ cells for autologous transplant and 4×10^6/kg of recipient bodyweight for allogeneic transplant [4,14]. The required number of CD34+ cells for allogeneic transplants is increased with increased human leucocyte antigen (HLA) disparity between donor and recipient or if a tandem transplant or graft manipulation is contemplated. The maximum number of cells to be infused is not defined. However, in the autologous setting, harvesting of much higher cell numbers is not justified by improvement of clinical outcome. Infusion of very high numbers of allogeneic cells increases the risk of extensive chronic graft-versus-host disease (GvHD).

The timing of G-CSF treatment plays a part in the mobilisation dynamics. Autologous stem cell collection efficacy is higher when G-CSF is administered three hours before apheresis rather than the evening before [5], and some unrelated donor registries split the G-CSF into two doses per day, including a dose on the morning of apheresis. Serial measurement of peripheral blood CD34+ cell count in autologous donors is usually obtained as soon as their total leucocyte count approaches 1×10^9/L. Collection, started at a level of 20 CD34+ cells/μL, gives the best yield. Healthy donors usually follow a more predictable course, with peak mobilisation at day 5, after four G-CSF injections. Some donors require further injections, either because of delayed mobilisation or an initial low cell collection.

Collection of HPC, Apheresis is a technically challenging procedure. Machine efficiency is measured by the percentage of CD34+ cells that can be collected at a specific peripheral CD34+ cell count, and is enhanced by processing more volumes of donor blood quickly to avoid inconvenience to the donor. Selective machines avoid contamination of HPC, Apheresis by platelet and red cells. This is important not only to improve stem cell cryopreservation and reduce infusion complications, but also to avoid thrombocytopenia in the donor.

Apheresis units should observe good manufacturing practice (GMP) and qualify new machines against published data and existing equipment to ensure new technologies meet international standards for donor safety and product specifications [15]. Validation is particularly important for those donor groups such as children or heavily pretreated autologous patients, who tend to mobilise poorly.

Other important operational features of apheresis machines include:

- Volume of the collected product, as smaller volumes are easy to cryopreserve, require a smaller storage space and are associated with less dimethylsulfoxide (DMSO) infusion toxicity.
- Minimal contamination of the final product with granulocytes, which may cause infusion-related adverse reactions.
- Low extracorporeal volumes, so reducing transient anaemia and hypovolaemia in small subjects and children and averting the need to prime with blood.
- Processing higher volumes of blood to improve the yield of CD34+ cells.

Several machines, such as COBE Spectra Optia, Amicus and COM.TEC, can collect stem cells with different efficiencies and selectivity. Spectra Optia and Amicus are automated and have small end-harvest and

extracorporeal volumes. Spectra Optia has a larger market share and is commonly used in the UK.

A total of 2–3 patient blood volumes are usually processed by the apheresis machine at each leucocytapheresis procedure. Large-volume leucocytapheresis (processing of 3–6 blood volumes over a longer period of time or by increasing the blood flow into the apheresis machine) may collect more CD34+ cells, reduce the number of leucocytapheresis procedures required and limit exposure to G-CSF. However, it is associated with more donor inconvenience, citrate toxicity and platelet loss. It has been used successfully for allogeneic donors and for poor mobilising autologous donors [4,5,16].

Lymphocyte donations are regularly collected from allogeneic donors for posttransplantation therapy (see Chapter 44). Recently, autologous lymphocytes are also collected for CAR-T cell therapy (see Chapter 45) and the success of collecting enough cells depends on planning the optimal timing for apheresis. Patients with progressive disease have a limited timeframe when effective apheresis collection can be safely undertaken [17].

Box 42.2 Possible mechanisms of action of plasma exchange.

- Removal of pathogenic antibodies.
- Sensitisation of antibody-producing cells to immunosuppressant and chemotherapeutic agents.
- Removal of pathogenic immune complexes that could prevent splenic blockade and hence improve monocyte/macrophage functions.
- Removal of cytokines and adhesion molecules.
- Replacement of missing plasma component (e.g. thrombotic thrombocytopenic purpura).
- Alteration of the cellular immune system. Observed alterations include:
  - Changes in lymphocyte numbers and distribution (decline in B cells and increase in T cells).
  - Changes in natural killer (NK) cell numbers and activity.
  - Increase in T-suppressor or T-regulatory cell function.
  - Shift from Th2 to Thl predominant pattern.

Source: Adapted from Reeves and Winters 2014 [18].

Plasma Exchange

Plasma exchange is an effective treatment for many immune conditions by removing or reducing a pathological substance or substances, for example immunoglobulins in hyperviscosity or myasthenia gravis. However, the response of diseases such as multiple sclerosis, not primarily mediated by autoantibodies, to plasma exchange has suggested other possible mechanisms of actions of this treatment [18]. These are summarised in Box 42.2.

The removed plasma is usually replaced with human albumin solution (HAS) of 4.5%. (In the USA, it is called human serum albumin [HSA] and is usually 5%.) Up to one-third of the exchange volume can be replaced by normal saline if the patient's starting albumin level is normal; otherwise, hypotension and/or peripheral oedema may follow. HAS provides the necessary oncotic pressure with fewer allergic reactions and an impressive safety record with regard to infection transmission. In other clinical scenarios, the exchange process is required to replace necessary plasma constituents [19]. In thrombotic thrombocytopenic purpura (TTP), for example, plasma exchange removes autoantibodies to the von Willebrand factor (vWF)–cleaving protease (ADAMTS13) and the associated ultra-large von Willebrand factor multimers, and replaces ADAMTS13 and vWF in fresh frozen plasma (FFP) [20,21]. Solvent detergent plasma is the recommended replacement fluid for TTP in the UK, while in the USA this product, though approved for use, is not currently widely utilised. Clotting factors may also require replacement during plasma exchange. A therapeutic dose of FFP (10–15 mL/kg) may be included as

the final replacement fluid where repeated exchange with albumin has depleted clotting factors in patients at high risk of bleeding.

Plasma exchange treatment plans specify the volume and timing of procedures to ensure efficiency. An exchange of 1.0–1.5 of the patient's plasma volume will exchange between 63% and 78% of their plasma and is therapeutically effective in most situations. Larger-volume exchange is inconvenient and expends replacement fluid with little extra benefit (Figure 42.1) [22]. The frequency and total number of exchanges depend on the disease being treated and on the patient's response. Hyperviscosity, TTP and Goodpasture syndrome require daily exchanges; other conditions may respond to a less intensive course of treatment, such as 5–7 exchanges over 10–14 days.

Criteria to monitor response to treatment should be agreed early in the treatment plan to avoid undertreatment, overtreatment or the continuation of ineffective treatment. TTP is monitored by measuring the platelet count and other parameters of haemolysis, while Guillain–Barré syndrome and myasthenia gravis are assessed by clinical neurological improvement.

The evidence base for the effectiveness of plasma exchange is expanding. The American Society for Apheresis (ASFA) regularly publishes a guidelines document with assignment of ASFA category (I to IV) and recommendation grade for different diseases [23]. Box 42.3 lists some of the category I indications where plasma exchange is considered first-line therapy. However, plasma exchange is also used for category II indications, where it is considered a second-line therapy for severe cryoglobulinaemia, acute relapsed multiple sclerosis, neuromyelitis optica, thyroid storm and many others [23].

Although large, randomised trials support the use of plasma exchange in the treatment of Guillain–Barré syndrome, intravenous immunoglobulin (IvIg) is equally effective and is usually a first-choice therapy. However, either of the two treatment modalities can be used if the other fails. Chronic inflammatory demyelinating polyradiculoneuropathy also responds to both plasma exchange and IvIg and the former can be used for maintenance treatment.

In myasthenia gravis, plasma exchange has a clear therapeutic effect, but the disease control is temporary and may be followed by a rebound. Here, plasma exchange is used to treat emergencies such as respiratory failure or swallowing difficulties and to prepare patients for thymectomy. Plasma exchange must be

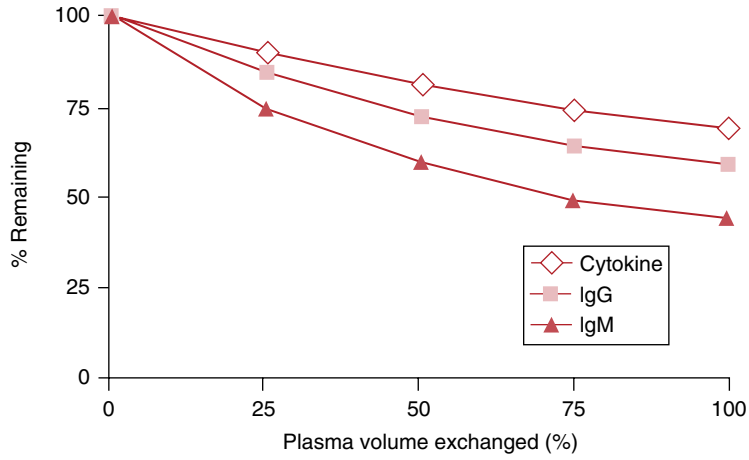

Figure 42.1 Kinetics of plasma exchange. Ig, immunoglobulin. *Source:* El-Ghariani and Unsworth 2006 [22]. Reproduced with permission of Royal College of Physicians.

Box 42.3 Disorders for which plasma exchange is accepted as first-line therapy, either as a stand-alone treatment or in conjunction with other modes of treatment (American Society for Apheresis category I indications).

Haematological Disorders
Hyperviscosity in hypergammaglobulinaemia
Thrombotic thrombocytopenic purpura
Thrombotic microangiopathies due to factor H autoantibody

Neurological Disorders
Guillain–Barré syndrome
Chronic inflammatory demyelinating polyradiculopathy
Myasthenia gravis
N-methyl-D-aspartate receptor antibody encephalitis
Paraproteinemic demyelinating neuropathies

Transplantation
ABO-incompatible liver or kidney transplantation
Antibody-mediated kidney transplant rejection

Other Immune/Metabolic Disorders
Goodpasture syndrome
Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis with pulmonary haemorrhage
Catastrophic antiphospholipid syndrome
Fulminant Wilson disease

Source: Adapted from the American Society for Apheresis guidelines [23].

accompanied by an appropriate immunosuppressive regime for long-term benefit.

Paraproteinaemia causing clinically evident and progressive hyperviscosity syndrome is a medical emergency requiring urgent plasma exchange to reduce the responsible paraprotein. IgM is mostly intravascular and therefore most likely to cause hyperviscosity. IgA and IgG3 tend to aggregate and may also be associated with hyperviscosity. One to three treatments will usually alleviate symptoms long enough for chemotherapy to take effect. These patients are often severely anaemic, but transfusion should be delayed until the viscosity has been lowered, as a rise in haematocrit can exacerbate their symptoms.

Plasma exchange can also be lifesaving in fulminant cryoglobulinaemia. Replacement fluids should always be warmed. The cause of the cryoglobulinaemia must be determined and definitive chemotherapy instituted if appropriate.

Plasma exchange plays a limited role in the treatment of autoimmune blood cytopenia; however, it is the treatment of choice for TTP and should be started as soon as the diagnosis is suspected [20,21]. Daily plasma exchange is needed for at least two days after the platelet count has returned to normal (i.e. $> 150 \times 10^9$/L) and lactate dehydrogenase (LDH) is within the normal range. Plasma infusion can also be used to treat TTP if plasma exchange is not immediately available. Plasma exchange is also effective in the management of thrombotic microangiopathies due to factor H autoantibodies or because of ticlopidine use.

Plasma exchange is required as an adjuvant therapy in anti-glomerular basement membrane disease (Goodpasture syndrome). Recently, the results from a large randomised controlled trial (RCT) have shown that plasma exchange benefits patients with diffuse alveolar haemorrhage in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis [24]. These patients should not be overloaded with replacement fluids to avoid provoking further bleeding. Plasma exchange is effective, in combination with steroids and anticoagulation, in the therapy of catastrophic antiphospholipid syndrome and in selected patients with systemic lupus erythematosus [23]. Plasma exchange has no proven role in the management of uncomplicated rheumatoid arthritis or antepartum HELLP syndrome.

There have been several recent reports describing the use of plasma exchange in age-related disorders. A large sham-controlled RCT in patients with Alzheimer's disease showed a significant effect in patients

with moderate disease [25]. It remains unclear what the impact of such studies would be on the utilisation of plasma exchange in the future.

Red Cell Exchange

Red cell exchange involves the removal of a patient's red cells and concomitant infusion of allogeneic donor cells. This procedure, evolved as a manual procedure, can be performed by apheresis machines and is most commonly used to treat sickle cell disease. An isovolaemic automated procedure is important in preventing further complications and is widely accepted by patients [26]. The patient's haematocrit, the fraction of the patient's red cells to be retained after the exchange, the desirable final haematocrit and the haematocrit of the replacement fluid can be used to calculate the volumes of red cells to be removed and used as replacement. Most centres reduce postprocedure haemoglobin (Hb) S level to ≤ 30% to treat and prevent vaso-occlusive crises. Moreover, the final haematocrit following exchange is usually kept at ≤ 30% [27]. Higher haematocrits are associated with a reduction in oxygen delivery. Neurological events after partial exchange, usually for priapism, have been observed and are thought to be due to high-end Hb levels, a situation also known as ASPEN syndrome. Issues with venous access remain problematic and may reduce patients' compliance [27].

Although transfusion therapy is effective in primary stroke prevention, evidence for the use of red cell exchange in the management of other complications of sickle cell disease is limited [23]. Clinical guidelines suggest red cell exchange to treat acute chest syndrome, acute ischaemic stroke and acute transient ischaemic attack (TIA) by providing rapid reduction of sickle Hb while avoiding iron accumulation [27,28]. In the chronic/prophylactic setting, red cell exchange is mainly used for secondary and primary stroke prevention and the management of recurrent acute chest or recurrent painful crises [23,27,29].

Automated red cell depletion exchange uses isovolaemic haemodilution (IHD) to lower patients' haematocrit to an operator-specific level through replacement of a portion of their red cell mass with normal saline or albumin before performance of a standard exchange [30]. However, it may increase the risks of vasovagal events and nausea. It is not recommended in patients with low starting Hb, young age or low bodyweight [27], nor for treatment of an acute severe event. IHD may reduce the overall mass of red cells used and occasionally increase the interval between exchanges. However, convincing trial data to support the routine use of IHD are currently lacking.

Red cell exchange is only one aspect of the clinical care of sickle cell disease. A comprehensive approach to manage all clinical, social and psychological needs of this group of patients is required [29,31]. Phenotyped blood should be used when possible to reduce the risk of red cell alloimmunisation and patients should be monitored for iron accumulation.

Red cell exchange as an adjuvant therapy in patients with severe malaria appears to improve blood rheological properties, but it may not improve survival [23]. Absolute erythrocytosis causing hyperviscosity, thromboembolism or bleeding should be treated by tackling its primary cause and possibly by phlebotomy to maintain a normal haematocrit. However, erythrocytapheresis is also used to treat certain patients with polycythaemia, where removed red cells are replaced with albumin or saline to maintain isovolaemia. This procedure is particularly useful in patients with polycythaemia rubra vera, complicated by acute thromboembolism, severe microvascular complications or bleeding, especially if the patient is haemodynamically unstable. Erythrocytapheresis is also used to treat hereditary hemochromatosis [23].

Extracorporeal Photochemotherapy

Extracorporeal photochemotherapy (ECP) collects a patient's mononuclear cells (MNC) and exposes them to ultraviolet A (UVA) light in the presence of photoactivating agents such as 8-methoxypsoralen (8-MOP). This induces immunomodulation, which can be beneficial for some immune-mediated disorders. The mechanism of action of ECP is not fully understood [32], but it induces lymphocyte apoptosis, which leads to changes in cytokine secretion patterns, more tolerant antigen-presenting cells (APCs), induction of T-regulatory (T-reg) cells and suppression of CD8+ effector cells.

ECP is thought to induce an immune response against cutaneous lymphoma. Interestingly, ECP does not lead to an increased incidence of opportunistic infection, which is particularly useful in patients with extensive skin lesions. ECP has been an established therapy for cutaneous T-cell lymphoma and acute and chronic GvHD [32,33]. It also has a role in the prevention of sold organ transplant rejection, treatment of scleroderma, Crohn's disease, type I diabetes and other immune-mediated skin disorders [34].

Extracorporeal photochemotherapy can be achieved by several methodologies [32]. In one-step methods, MNCs are collected using a specialised cell separator such as the Therako Cellex system (most commonly used in the UK and USA) or Amicus Blue (only approved in the European Union). These machines deliver a calculated UVA radiation dose into the MNC suspension pretreated with 8-MOP, before returning the cells to the patient's circulation. Heparin, and less commonly anticoagulant citrate dextrose solution (ACD-A), is used as an anticoagulant. Alternatively, ECP can be completed using two-step methods, which are composed of a combination of a cell separator to collect leucocytes, 8-MOP is added to the apheresis product and the suspension is then exposed to UVA using an irradiation source (UV light box), such as the UV-matic irradiator (Vilber-Lourmat, Marne-la-Vallée, France), and then reinfused. This practice is used in Europe, but strict adherence to GMP regulations for reinfused products is required. Regardless of the methodology used, ECP is usually well tolerated and has no serious side effects [32]. ECP is contraindicated in the presence of psoralen hypersensitivity.

Guidelines to direct the ECP are available [32–34]. ECP is a first-line therapy for patients with advanced mycosis fungoides and Sézary syndrome. Patients receive one treatment cycle (i.e. one ECP procedure per day on two consecutive days) every 2 weeks for the first 3 months and then one cycle every 3–4 weeks. ECP is a second-line therapy in patients with steroid-dependent, steroid-intolerant or steroid-resistant chronic GvHD and those with recurrent infections. Weekly ECP cycles are recommended for the first 3 months and then tapered depending on clinical response. Patients with acute GvHD who show progression after 3 days of steroid therapy or lack of response after 7 days of steroid therapy should receive ECP as second-line therapy. Weekly cycles are required until a response is achieved. More recently, ECP has been combined with ruxolitinib (a selective Janus kinase 1/2 inhibitor) to treat severe refractory acute and chronic GvHD, with encouraging results [35,36]. Further research should be directed to better understand its mode of action, search for optimal duration and frequency, and how to integrate ECP with other immunosuppressor regimes to achieve better disease control.

Apheresis Applications for COVID-19 Patients

The COVID-19 pandemic has affected, in one way or the other, most if not all healthcare systems. Some apheresis units have

noticed a reduction in numbers of patients referred for treatment. This may reflect changes in healthcare priorities and/or reluctance of patients to attend hospitals. Apheresis technologies have been used, on a large scale, to collect convalescent plasma to treat hospitalised COVID-19 patients. Randomised trials have not so far shown the clinical benefit of such treatment, although trials are continuing to test therapy for immunocompromised patients and for early therapy of vulnerable patients. Plasma exchange may be useful second-line therapy for vaccine-induced thrombosis and thrombocytopenia (VITT) if alternative anticoagulation and IvIg are not effective [37].

Sickle cell disease predisposes patients to severe COVID-19 infection and in the UK such patients are considered high risk for COVID-19 complications. COVID-19 infection could trigger acute chest syndrome and there are several case reports of such patients being treated by red cell exchange [38]. Finally, autologous and allogeneic stem cell and lymphocyte donors must test negative for COVID-19 before apheresis collection can commence. Regulators and professional bodies are regularly providing advice on COVID-19 management of cell therapy donations. The European Society for Blood and Marrow Transplantation has published its recommendations [39] and keeps them updated on its website (www.ebmt.org).

Complications of Therapeutic Apheresis

Complications occur in up to 10% of procedures; most are mild, but rarely serious complications, including deaths, have been reported. Given the advances in technology, machine-related problems such as air embolism are unusual. Failure of the machine that will prevent red cell return can result in red cell loss of up to 150 mL of blood in newer instruments and up to 350 mL in older instruments. Central catheter-related complications, such as pneumothorax, internal bleeding, thrombosis and infections, can be serious and are the main concern with regard to apheresis complications. Allergic reactions to replacement fluids are uncommon, but can be significant. These include anaphylactic reactions, hypotension and urticarial rashes. Reactions to HAS are now rare, as the preparations contain lower amounts of significant contaminants than previously, especially of vasoactive kinins. HAS essentially carries no risk of infection and does not increase the citrate return. Dilution of coagulation factors can occur following repeated plasma exchanges and may require the addition of FFP to the replacement fluid. FFP poses the risk of blood-borne infection (although virally inactivated products are now available) and allergic reactions, and contributes to the citrate load, as FFP contains approximately 14% citrate anticoagulant by volume. Side effects of the citrate anticoagulant, almost universally used, are particularly common. These result from hypocalcaemia and include paraesthesia (digital and perioral), abdominal cramps and, rarely, cardiac dysrhythmias and seizures. Citrate toxicity usually responds to simple measures such as slowing the flow rate and providing extra calcium orally. Intravenous calcium may be required, especially in patients receiving a significant citrate load such as during HPC collection. Patients with renal failure who are receiving large amounts of citrate during plasma exchange may develop a profound metabolic alkalosis. Patients receiving repeated treatments over a long period of time can lose significant quantities of calcium.

Complications during therapeutic apheresis may arise from underlying pathology or co-morbidity. It is important that the clinical status is assessed prior to exchange. Where risks are increased but benefit is likely, a suitable location for the procedure such as a high-dependency unit may be required.

KEY POINTS

1) A physician experienced in the use of cell separators should assess the patient's need to have a therapeutic apheresis procedure, taking into consideration the potential risks and inconvenience.
2) Adequate vascular access is crucial. Central venous catheterisation needs to be undertaken by trained staff to minimise risks to patients.
3) Granulocyte colony-stimulating factor (G-CSF) with or without chemotherapy is currently the gold standard for haematopoietic progenitor cell mobilisation. Donors who prove to be hard to mobilise may respond favourably to the addition of a CXCR4 antagonist (plerixafor) to the G-CSF mobilisation protocol.
4) Human albumin solution (4.5%) is the most commonly used replacement fluid for plasma exchange. Occasionally plasma, possibly solvent detergent product, is needed.
5) Plasma exchange causes several changes to the immune system and the mechanisms of action that mediate these changes require further research.
6) Red cell exchange is regularly used to treat some acute and chronic complications of sickle cell disease. Further research and trial evidence are required to guide this practice.
7) Photopheresis induces immunomodulation without systemic immunosuppression and is indicated for treatment of specific indications of cutaneous T-cell lymphoma, graft-versus-host disease and solid organ transplant rejection.

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43 Haemopoietic Stem Cell Processing and Storage

Akash Gupta and Ronan Foley

Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

Background

Once exclusively obtained from the posterior pelvis of a suitable donor under general anaesthesia, haemopoietic progenitor cells (HPCs) are now routinely collected from peripheral blood as well as from the umbilical cord and placenta post delivery. Recognition that haemopoietic growth factors (i.e. granulocyte colony-stimulating factor, G-CSF), administered alone or following chemotherapy, result in mobilisation of HPCs into peripheral blood has had a profound impact on stem cell collection, both for autologous transplantation and for healthy allogeneic stem cell donors. Expression of the CD34+ antigen can be used to identify HPCs. Functional evaluation of colony-forming units (CFUs) of myeloid, erythroid, megakaryocytic and long-term culture-initiating cells may be useful functional assays that can complement immunophenotypic analysis.

The International Society for Blood Transfusion (ISBT) has proposed (ISBT 128 nomenclature) terminology to describe HPC and other cell-based human products derived from a bone marrow harvest (HPC, Marrow), mobilised apheresis peripheral blood (HPC, Apheresis), umbilical cord (HPC, Cord Blood) or from steady-state apheresis for donor lymphocyte infusion (DLI) (Mononuclear cells, Apheresis) (Table 43.1). Each source exhibits different biological properties and graft composition, which offers advantages and disadvantages for specific types of clinical transplant procedures. Cytokine-mobilised apheresis products contain a higher number of CD34+ progenitors, which may be ideal for reduced-intensity allogeneic and autologous transplants. Apheresis also harvests more T lymphocytes, which may increase chronic graft-versus-host disease (GvHD) [1]. Cord blood may have fewer progenitor cells, but compensates by containing cells with a higher proliferative potential and a lower risk of GvHD [2].

Transplant Procedures

Autologous Stem Cell Transplant

Expanding indications support the use of autologous stem cell transplant (SCT) in a variety of clinical settings, including multiple myeloma (single or tandem) and relapsed Hodgkin and non-Hodgkin lymphoma (B and T aggressive histology lymphoma, mantle

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Table 43.1 Human haemopoietic progenitor cells (HPCs).

Name	Donor	Options	Storage
HPC, Marrow Collect 10–15 mL/kg recipient weight with maximum 20 mL/kg donor weight Dose 2–4 × 10^8/kg recipient weight	Matched related donor Matched unrelated donor Autologous (rare)	Standard intraoperative marrow harvest adults or children	Usually infused within 6 hours following collection Cryopreservation post buffy coat concentration
HPC, Apheresis Process 12–20 L of donor blood	Autologous patient (common)	Apheresis product collected following a stem cell mobilisation agent (G-CSF, pegylated G-CSF, GM-CSF, plerixafor) +/– Chemotherapy	Auto SCT – cryopreserved
	Matched related donor	Mobilise with 5–10 μg/kg G-CSF	Allo SCT – infused following collection or cryopreserved
	Matched unrelated donor	Mobilise with 5–10 μg/kg G-CSF	
	Haploidentical donor	Mobilise with 16 μg/kg G-CSF	May include CD34+ selection, T-cell depletion and cryopreservation
MNC, Apheresis or T Cells, Apheresis Donor lymphocyte infusion	Matched related donor Matched unrelated donor	Same donor as original HPC product – steady state Dose = CD3+/lymphocyte/kg recipient weight	Graduated doses, first dose following collection, later doses cryopreserved
HPC, Cord	Cord blood approximately 100 mL	Consenting parent(s) > 3.0 × 10^7/kg recipient weight	Red cell depleted cryopreserved
	Matched unrelated donor	*In utero* *Ex utero*	Final volume is 20 mL with 5 mL of DMSO/dextran solution

DMSO, dimethyl sulfoxide; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; MNC, mononuclear cell; SCT, stem cell transplant.

cell lymphoma, Burkitt and follicular lymphoma). Autologous SCT is also a therapeutic option for patients with gonadal or retroperitoneal germ cell tumours refractory to cisplatin-based chemotherapy. At present autologous progenitor cells are obtained by leukapheresis. Use of HPC, Apheresis reduces the time to engraftment and leads to shorter hospitalisation, reduced transfusion support and reduced use of antimicrobials [3], all leading to cost reductions.

Allogeneic Stem Cell Transplant

Allogeneic SCT involves replacement of a diseased bone marrow with haemopoietic elements from a healthy donor. Engraftment of both HPCs as well as donor immune T lymphocytes is essential for long-term haemopoiesis and disease control. Donor lymphocytes contribute to both graft-versus-leukaemia (GvL) as well as GvHD. Shifting the balance of therapeutic efficacy solely from stem cell replacement to maintenance of a transplanted donor immune system has led to reduced-intensity conditioning (RIC) allogeneic transplants.

Allogeneic donors may be a sibling, or a donor obtained from a bone marrow registry. Matches are based on the human leucocyte antigen (HLA) system, comprised of genes on chromosome 6. The major histocompatibility complex (MHC) is made up of two basic classes involved in antigen presentation and immune activation. MHC class I includes HLA-A, HLA-B and HLA-C, whereas MHC class II includes HLA-DR, HLA-DQ and HLA-DP. Proteins encoded by HLA define self and directly instruct the immune system to recognise self-versus non-self. HLA typing previously employed simple serological testing (antibody based) to provide low-resolution (LR) typing. Although previously useful in the related setting, most HLA laboratories now routinely perform high-resolution (HR) molecular typing for both related and unrelated donor typing.

If a complete match from an allogeneic or unrelated donor cannot be found, the remaining options include haploidentical stem cell transplantation or allogeneic transplant using cryopreserved cord blood. Haploidentical SCT involves a family member with only a partial HLA match (4/8). One advantage of using a haplo approach is an expanded donor selection pool, which would include parents, siblings, children and other first-degree relatives. However, achieving engraftment with haploidentical donors is a major challenge. Due to these significant HLA barriers, large-volume CD34+ products may be rigorously purified (T depleted). This may result in lasting immune deficiency, with a high risk of fulminant infection (viral, cytomegalovirus [CMV], Epstein–Barr virus [EBV]) or relapse. *In vivo* T-cell depletion may be achieved with administration of cyclophosphamide following stem cell infusion, which is a technique that has gained recent popularity in haploidentical transplants [4]. Other anti-GvHD therapies include anti-thymocyte globulin (ATG), G-CSF and triple GvHD prophylaxis [5].

Donor Lymphocyte Infusions

Apheresis from an HPC donor in an unstimulated state is called MNC-Apheresis or T-Cell Apheresis (CD3+ content known). These DLI products may be used to boost a mixed chimerism to full donor chimerism after allogeneic HPC transplant or as pre-emptive therapy for treatment of an early relapse via a direct GvL effect. Given a risk of severe GvHD, graduated doses of donor T cells are often administered over time. Lymphocyte content is calculated using automated or manual differential as well as flow cytometry (CD3+). The laboratory will aliquot doses (defined by institution or protocol) based on the number of CD3+ T cells $\times 10^6$/kg of recipient weight, often giving the first dose fresh and cryopreserving other doses.

Haemopoietic Progenitor Cell Products

Bone Marrow

Use of bone marrow (HPC, Marrow) remains a mainstay of treatment for paediatric or patients with aplastic anaemia. Collecting

bone marrow involves placing a suitable donor under general anaesthesia and aspirating 10–15 mL/kg recipient weight (maximum 20 mL/kg donor weight) from both posterior iliac crests. Collected fresh product (mixed with ACD/heparin) is passed through 500 and 200 μm filters to remove bone and other debris prior to infusion or further processing. The target nucleated cell dose (automated counter) is 3–5 × 10^8/kg recipient weight. Use of marrow CD34+ enumeration suggests that a CD34+ cell dose > 3.0 × 10^6/kg correlates with improved recovery and 5-year survival, while < 1.2 × 10^6/kg correlates with inferior recovery [6,7].

Peripheral Blood

Mobilisation of CD34+ progenitor cells into blood with collection by leucapheresis (HPC, Apheresis) is the method of choice for autologous SCT. This procedure is based on obtaining sufficient CD34+ progenitor cells (defined as a minimum of 2.0 × 10^6 CD34+ cells/recipient weight and an optimal of 5.0 × 10^6 CD34+ cells/recipient weight). Lower doses of infused CD34+ cells can result in delayed or failed platelet engraftment. Case-by-case decisions are made to proceed with auto SCT when < 2.0 × 10^6/kg CD34+ cells based on the clinical situation and stability of the underlying disease. For patients undergoing auto SCT, two mobilisation strategies can be employed: either growth factor(s) alone or growth factors that follow administration of chemotherapy (chemo-mobilisation).

In some patients, G-CSF and chemotherapy still result in suboptimal stem cell yields. Plerixafor (AMD3100) is an immunostimulant drug that can antagonise the binding of the chemokine stromal cell–derived factor-1 (SDF-1) to its cognate receptor CXCR4, and in doing so it rapidly and reversibly mobilises haemopoietic stem cells into the peripheral circulation. Its activity synergises with G-CSF. Utilising both G-CSF and this CXCR4 antagonist can salvage those who fail G-CSF mobilisation alone.

Healthy allogeneic donors may also be asked to provide mobilised peripheral blood progenitors collected by leucapheresis. Administration of G-CSF alone is the currently accepted strategy for mobilisation of normal healthy donors. Use of HPC, Apheresis as opposed to HPC, Marrow appears to improve the time to haemopoietic recovery and offers a greater GvL effect [7], but carries a potentially higher risk of chronic extensive GvHD [8].

Umbilical Cord Blood

Characteristics of banked cord products include highly functional HPCs, less CMV contamination and a lower risk of GvHD. It is generally accepted that the kinetics of haemopoietic recovery are significantly slower. This may relate to fewer and less mature HPCs present within the product. A minimum target of approximately 3.0 × 10^7 nucleated cells per recipient weight per unit of cord blood is suggested. A higher dose may be considered, depending on HLA disparity. Measurement of CD34+ cells/recipient kg weight may be more informative. The mean collection volume for a cord sample is approximately 100 mL (50–200 mL) including anticoagulant [9].

Several techniques for cord blood collection may be performed either prior to or following delivery of the placenta. Closed-system collection techniques have decreased rates of bacterial contamination. Cells can be stored in a smaller volume by immediately removing plasma and red blood cells. Characterisation of the cord unit includes volume, weight, total nucleated count, CD34+ cell count, colony-forming analysis, ABO/Rh and HLA typing, full panel transmissible disease testing and haemoglobin electrophoresis [10]. *In vitro* analysis has suggested reasonable viability to as long as 15 years, perhaps longer [11]. To hasten time to reconstitution in larger adult recipients,

double umbilical cord blood can be considered [12].

Haemopoietic Progenitor Cell Product Assessment and Specialised Procedures

See Table 43.2.

CD34 Enumeration

Flow cytometry on a fresh HPC product provides CD34+ enumeration in approximately one hour. Given the importance of accurate CD34+ enumeration, the procedure should follow a standardised and validated methodology (i.e. ISHAGE guidelines for CD34+ cell

Table 43.2 Stem cell laboratory processing procedures.

Procedure	Methods	Indication
Red cell depletion of HPC, Marrow	Semi-automated – Cobe 2991 cell processor with or without HES Manual centrifugation	Major ABO/other antigens Cryopreservation of HPC, Cord Blood
Plasma depletion of HPC, Marrow	Semi-automated – Cobe 2991 cell processor Manual centrifugation	Minor ABO mismatch
Buffy coat concentration	Centrifugation Semi-automated – Cobe 2991 cell processor	Volume reduction Cryopreservation of HPC, Marrow
Sterility	Bacterial and fungal detection Investigational products (mycoplasma, adventitial virus, endotoxin)	HPC products pre cryopreservation and post thaw Cryoprotectant solutions
Viability	Dye exclusion (TB), fluorescence microscopy 7-AAD – flow cytometry	Products used after more than 2 years of storage
CD34/CD3 enumeration	Flow cytometry (i.e. ISHAGE)	HPC, Apheresis HPC, Marrow HPC, Cord (optional) MNC, Apheresis
Functional HPC assays	CFU assays, LTC-IC	Viability post long-term storage Viability post investigative procedure (purging)\ Validation of new procedure to document HPC loss Assess stored cryopreserved product post 'warming event'
CD34 enrichment	Immunomagnetic bead-based separation	Related haploidentical SCT 'purge' technique Selected cases (GvHD prophylaxis) Clinical trial
T-cell depletion	Antibody based +/– toxin Elutriation	Investigational product Selected cases Clinical trial
Cryopreservation	DMSO, HES/DMSO controlled-rate freezing or freeze in –80 °C Liquid nitrogen storage below –150 °C	Option for all HPC products

7-AAD, 7-amino actinomycin D; CFU, colony-forming unit; DMSO, dimethyl sulfoxide; GvHD, graft-versus-host disease; HES, hydroxethyl starch; HPC, haemopoietic progenitor cell; ISHAGE, International Society of Hematotherapy and Graft Engineering; LTC-IC, long-term culture-initiating cell; MNC, mononuclear cell; SCT, stem cell transplant; TB, trypan blue.

determination by flow cytometry [13]). A CD34+ enumeration kit includes CD45-FITC/CD34-PE, isotype control PE, stem cell microbeads (known concentration/μL), lysing solution (ammonium chloride) and a viability dye 7-amino actinomycin D (7-AAD). HPC samples stored at 18–20 °C should be processed within a few hours and samples kept overnight should be stored at 2–6 °C. Total viable CD34, apoptotic and necrotic cells can all be measured with calculations based on the product volume.

Enumeration of the peripheral blood CD34+ (expressed per μL) prior to collection may be instructive and predictive.

Viability Assays

Trypan blue (TB) is a simple exclusion dye test indicating viability. Cells that fail to exclude dye are considered non-viable. Use of fluorescent stains with dark-field microscopy may reduce background staining. 7-Aminoactinomycin (7-AAD) is a fluorescent chemical with affinity for guanine-cytosine (GC)-rich DNA. Non-viable cells lack membrane integrity and will take up 7-AAD, which can be measured by flow cytometry. If performing viability for cryopreserved HPC product, it is important to keep in mind that small aliquots will have different cooling properties that may diminish viability. So viability results from a cryovial are simply an estimate of viability for an actual product contained in a bag.

In vitro Haemopoietic Progenitor Cell Assays

Functional analysis of HPCs can be performed using cells grown in semi-solid methylcellulose (MC) to identify CFUs. Resultant CFU-erythroid, CFU-granulocyte, CFU-megakaryocyte and CFU-mixed (CFU-GEMM) colonies help to characterise the short-term multipotency of a given HPC product. These assays are time consuming (two weeks) and do not provide real-time information for products that are administered shortly after collection. Facilities managing HPC, Cord products that are stored frozen over long periods may offer colony assay results together with CD34 content. These assays are useful in the evaluation of long-term storage and for validation of newer cryopreservation strategies.

Sterility Testing

At a minimum, sterility testing must be performed post processing, and should be suitable to detect clinically significant bacteria and fungal contamination of an HPC product. It is our preference to collect samples for culture when the product arrives in the laboratory and after each step of processing. Paediatric bottles can be used to minimise the volume of sample removed from the product. Cultures should be performed (1) at the end of HPC product collection; (2) at the end of processing for cryopreservation; and (3) after each reprocessing step (washing cells, manipulation on cell processor, post CD34+ selection). Cultures may also be obtained from each bag at the time of reinfusion.

ABO Incompatibility

HLA-matched HPC SCT can proceed even if the blood groups of donor and recipient do not match. There are three types of ABO mismatch, each with its own interventions, which may need to occur before the transplant product can be infused [14]. ABO major mismatch results when the recipient's plasma contains a potent ABO antibody directed against the donor's red cells. HPC, Apheresis products have a haematocrit of 5–10%, whereas the red cell content of HPC, Marrow haematocrit is much higher, ranging from 25% to 30%. Significant intravascular lysis of red cells will cause a haemolytic transfusion reaction if the product is infused without a reduction in the level of the ABO antibody. Recipients receiving an HPC, Marrow who have an ABO antibody titre against donor's red cells greater than 1 : 16 may be pretreated by performing several apheresis procedures to replace their plasma with 5% albumin. If the albumin apheresis does not lower the titre sufficiently, the titre

of antibody can be further reduced by infusing plasma containing a soluble ABO substance that matches the antibody of interest. These measures are generally sufficient to allow safe infusion of HPC, Marrow, but significant residual antibody after other interventions may require processing of the HPC, Marrow to remove most of the red cell content. Red cell depletion of the bone marrow is most commonly performed using a blood processor such as the Cobe® 2991 or Optia® (TerumoBCT, Lakewood, CO, USA), with or without the addition of a regimenting agent like hydroxethyl starch (HES). Red cell content at completion of processing should be as low as possible while minimising loss of progenitor cells.

An ABO minor mismatch results when the donor's plasma contains a potent antibody directed against the recipient's red cells. The need for intervention is less common in this setting, as the ratio of antibody to red cell antigen is much lower, but it is wise to assess the level of donor antibody against the intended recipient's red cells. The presence of an antibody with a titre above 1 : 256 may necessitate group O red cell exchange of the recipient if the product is HPC, Apheresis. HPC, Marrow should undergo plasma depletion to remove at least 80% of antibody, either through centrifugation or in semi-closed mode using a Cobe 2991 or similar blood processor.

An ABO major/minor mismatch occurs when both the donor's plasma and the recipient's plasma have antibodies against each other, for example with a blood group B donor and a blood group A recipient. Based on the antibody titres, interventions may be required to reduce the risk of haemolysis, as described above.

CD34+ Enrichment

CD34+ enrichment of HPC products is performed for a variety of reasons: haploidentical transplant, reduction of potential tumour burden in autologous HPC products and providing a T-cell depleted product to a recipient at high risk of GvHD. Both HPC, Apheresis and HPC, Marrow can be enriched for CD34+ cells, but the marrow product must first be processed to derive a buffy coat concentrate by reducing the overall volume and the red cell content. Commercially available CD34+ enrichment devices using monoclonal antibodies have proven highly effective. The CliniMacs^{TM} instrument from Miltenyi Biotec (Bergisch Gladbach, Germany) produces an extremely pure product (with an average 98% T-cell depletion) while recovering 65–75% of the initial CD34+ cell content.

T-Cell Depletion

Despite the use of potent immunosuppressive agents (i.e. methotrexate, cyclosporine), GvHD remains a common (up to 50%) complication for patients undergoing allogeneic SCT. GvHD is primarily mediated by T lymphocytes, which can be successfully removed from the graft prior to administration. T-cell depletion can clearly reduce GvHD, but also may hinder engraftment, increase the incidence of leukaemic relapse and the risk of infections, including posttransplant lymphoproliferative disorders. *Ex vivo* procedures to reduce T cells include physical separation by density gradient (counterflow centrifugal elutriation), depletion with lectins, cytotoxic drugs and the use of anti-T-cell antibodies (examples are CD2, CD3, CD5, CD8, CD25 and CD52) alone or in combination (complement, conjugated to toxin). Despite an ability to significantly eliminate T cells to as low as $< 1 \times 10^5$ CD3+ cells/kg recipient weight and attenuate acute GvHD, no differences in chronic GvHD, transplant-related mortality and disease-free survival have been proven to date [15].

Storage of Haemopoietic Progenitor Cell Products

Often, due to logistical reasons, the HPC product is stored for short periods of time (hours) in an unmanipulated liquid state.

The reported temperatures that are suitable for short-term storage range from 4 to 27 °C. An ambient temperature is often preferred for short-term storage of HPC, Marrow [16]; however, 'ambient' should be a specific temperature range, e.g. 18–22 °C. HPC collected by apheresis can be held at room temperature for 1–2 hours if further processing is to occur imminently. Otherwise, HPC should be stored at 4 °C when longer storage is required. There is a progressive loss of progenitor cells during non-frozen storage, with the rate of loss influenced by several factors, including cell concentration, quantity and type of other cells contained in the product, the storage bag and the storage temperature [17]. The leucocyte count should be diluted with donor plasma to $< 2 \times 10^8$/mL if overnight storage is planned.

The length of time the product can be stored should also be established by in-house viability measurements and an expiry date and time set for each type of product handled. Storage requirements should include designating a location dedicated to HPC products and a separate, clearly labelled location for any product that must be quarantined. A mechanism for monitoring and documenting temperature that includes both local and remote alarms must be in place. If the product is stored at 'ambient temperature', the temperature of the location of storage must be documented. There should be a posted contingency plan that deals with temperature outside the designated range or mechanical failure of the designated storage equipment.

Cryopreservation

The majority of allogeneic products are not cryopreserved. However, cryopreservation of allogeneic products may allow increased flexibility in the timing of the transplant related to the donor collection. In other cases, donor availability or a change in the recipient's condition may dictate that the collected product be cryopreserved. Virtually all products to be used for autologous SCT are cryopreserved to allow time for conditioning and clearance of chemotherapy drugs from the circulation.

Preparation of Products for Freezing

HPC products to be cryopreserved must be transported to the processing laboratory in a designated transport cooler that has been validated for transport time and temperature. The receiving staff must document the minimum/maximum and actual temperatures of transport and inspect the product for colour, leakage and correct labelling. All materials to be used in the cryopreservation process should have lot number and expiration date recorded. The product must be manipulated in a fully maintained and inspected biohazard safety cabinet. HPC, Marrow and HPC, Cord products require processing to reduce mature red cell content and volume reduction before cryopreservation processing can occur. In most instances the haematocrit of HPC, Apheresis is between 5% and 10%, and these products can be cryopreserved without removal of mature red cells. Plasma collected from the donor (via apheresis) or retained from red cell depletion (referred to as concurrent plasma) should always accompany the product to the processing laboratory in case there is a need to dilute the product.

Sterility testing must be performed on the product both on arrival and after the addition of the cryoprotectant solution. Once sterility samples are collected, samples should be drawn for a nucleated cell count and a CD34 assessment. Products with a nucleated cell count higher than 4.5×10^8/mL should be diluted with concurrent donor plasma.

Techniques for cryopreservation are designed to interfere with mechanisms that cause cell damage or death during the freezing process [18]. HPCs need to be protected from dehydration and ice crystal formation within the cell. Dimethyl sulfoxide (DMSO) is a 'penetrating cryoprotectant' that acts not only by slowing water absorption by the ice crystals, but also by rapid diffusion into the cell and so facilitating movement of water

out of the cell without excessive osmotic stress and before intracellular ice crystal formation can occur.

Freezing Cellular Products

The cooling rate should minimise ice formation potential and complement the cryoprotectant's adjustment of the solution's rate of cooling. The optimum concentration of DMSO to achieve good penetration of cells and moderation of the freezing point of the extracellular water is 10% volume. Reduced concentrations of DMSO (5%) can be used if DMSO is combined with a macromolecular cryoprotectant such as HES [19]. Cryoprotection can be accomplished by using macromolecules alone, but a 'combined' cryoprotectant seems to afford better cell recoveries than the use of macromolecules alone. This type of cryoprotectant solution is a complex blend of salts, sugars, DMSO and plasma proteins and requires careful preparation. Plasma proteins also have cryoprotectant properties and the addition of serum proteins as donor plasma or 5% human serum albumin to a cryoprotectant solution appears to improve HPC survival.

Red Cell Removal

Processing of products to remove mature red cells should be performed prior to cryopreservation for HPC, Marrow and HPC, Cord Blood, to limit infusion of free haemoglobin and renal toxicity. Large quantities of red cells can also cause clumping of the product during processing. Bone marrow product may be processed before cryopreservation, not only to remove red blood cells but also to eliminate fat and most of the plasma volume and so allow cryopreservation at a desirable cell concentration to optimise cell recovery, and to reduce the volume of the final product. Processing can be performed manually between 800 g and 1000 g or in a semi-automatic manner using a Cobe 2991 cell processor. Most laboratories freeze HPCs at concentrations between 1.0 and 5.0 $\times 10^8$/mL, but successful cryopreservation and recovery have been reported using concentrations as high as 8×10^8/mL and as low as 1×10^6/mL.

Most centres avoid the risks of large ice crystal formation by storing at or below –120 °C in mechanical freezers or in the vapour or liquid phase of nitrogen. Products and temperature-monitoring devices should be placed well below the rim of liquid nitrogen freezers to minimise the increase of temperature caused by opening the lid. Products exposed to frequent temperature change are at risk of progressive damage to stored cells and this can be reduced by placing products well below the rim of liquid nitrogen freezers, and by using aluminium storage canisters and frameworks to moderate thermal changes in the freezer. Products may be stored for at least 7 years when engraftment kinetics are identical to those seen after transplantation for the first half of the product stored for only 1–2 months [20].

Thawing of Cryopreserved Haemopoietic Progenitor Cells

Thawing can occur at the patient bedside or in the laboratory. Units should be transported in a liquid nitrogen dry shipper with continuous temperature monitoring to maintain the temperature below –120 °C. In most instances thawing is performed in a water-bath between 35 and 39 °C. If the cells are thawed too slowly there is risk of injury from ice recrystallisation; if the temperature is too high there is loss of viability or clumping of protein material within the bag.

Cryoprotectant can cause toxicity to the recipient, but if the dose of DMSO is carefully controlled it is not necessary to remove it prior to infusion. The DMSO dose should be limited to less than 1 g/kg of recipient weight in a 24-hour period. If the total amount of DMSO exceeds this limit, infusion should occur over two days. DMSO can be removed using serial dilutions of protein-based solution to avoid osmotic shock to cells. DMSO infusion can cause nausea, chills, cardiac arrhythmias, neurological symptoms and

respiratory arrest, but the majority of reactions are transient, and few patients require clinical treatment [21].

Quality Assurance

A quality programme defines the policies and environment necessary to attain acceptable outcomes and meet safety standards consistently. The components include standard operating procedures (SOPs) that address all activities; standardised and controlled labelling; documentation/record keeping that ensures traceability; personnel qualifications and training; building, facilities and equipment validation; environmental monitoring; regular auditing; and error and accident system/management. Regulatory authorities worldwide have placed major emphasis on the establishment of an effective quality programme along with strict compliance with best practices in clinical, collection and laboratory settings. At the processing laboratory the quality programme is how good manufacturing practices are instituted and followed throughout product manufacturing and manipulation.

KEY POINTS

1) Clinical indications for both autologous and allogeneic stem cell transplants are increasing.
2) Haemopoietic progenitor cells (HPCs) can be obtained from three sources: bone marrow, peripheral blood and umbilical cord blood. Characteristics of these products differ in terms of HPCs and other mature cells.
3) Validated methods for cryopreservation are a key requirement to ensure optimal graft performance following administration to a transplant recipient.
4) Accurate nucleated cell counting, CD34+ enumeration and sterility analysis before and after cryopreservation are essential.
5) Quality assurance testing of products at multiple stages of processing is essential to assure the safety, composition and potency of the product, and must form part of an established quality assurance and accreditation programme to guarantee high standards in HPC transplantation.

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44 Haematopoietic Cell Transplantation

Richard Salisbury[1], *Yisu Gu*[2,3,4], *Rachael Hough*[5] *and Robert D. Danby*[1]

[1] *Churchill Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, UK*
[2] *Department of Haematology, Churchill Hospital, Oxford, UK*
[3] *BRC Haematology Theme, Radcliffe Department of Medicine, University of Oxford, UK*
[4] *Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK*
[5] *University College London Hospital's NHS Foundation Trust, London, UK*

Haematopoietic stem cell (HSC) transplantation is an intensive and toxic treatment that offers the chance of cure for many malignant and non-malignant diseases of the haematopoietic system that cannot be adequately treated with chemotherapy or radiotherapy alone. Patients are given a course of conditioning chemo-radiotherapy followed by infusion of blood or bone marrow–derived cells that contain a sufficient dose of haematopoietic cells. These cells then engraft within the recipient and reconstitute the blood and bone marrow with donor-derived haematopoietic cells.

The recognition that radiation could permanently kill bone marrow function while other organs were largely unaffected or fully recovered suggested that bone marrow transplantation (BMT; also known as haematopoietic cell transplantation, HCT) might be feasible [1]. Initially, pretransplant conditioning chemo-radiotherapy was thought to provide 'space' for the incoming cells to engraft, as well as killing residual cancer cells. Therefore, *autologous* (HSC from the patient) and *allogeneic* (HSC from another individual) transplants were perceived as intensification of treatment with haematopoietic 'rescue' only. However, it became apparent that allogeneic HCT also produced an immune-mediated graft-versus-tumour (GvT) effect, since patients with chronic graft-versus-host disease (GvHD) had less relapse and improved disease-free survival [2]. Some patients with chronic myeloid leukaemia (CML), who relapsed after allogeneic HCT, could also return to full molecular remission by infusion of additional lymphocytes from the original donor (a donor lymphocyte infusion, DLI), further evidence of a GvT response [3].

Allogeneic HCT is therefore a combination of chemotherapy and/or radiotherapy with the donor-derived immune response, providing a major component in treating the original disease. As such, allogeneic HCT has a lower relapse rate, compared to autologous HCT, but is associated with a higher incidence of posttransplant infections and immune-mediated complications.

Principles of Haematopoietic Cell Transplants

Haematopoietic cell transplantation is used to:

- Enable intensification of chemotherapy and radiotherapy so that toxicity to the

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bone marrow is no longer the major limiting factor in determining outcome.
- Ensure complete engraftment of the donor marrow through immunosuppression of the host (patient), so permitting tolerance to develop.
- Promote a GvT effect.

Indications for Haematopoietic Cell Transplants

Haematopoietic cell therapy is used when conventional dose treatment has failed or is expected to have a high likelihood of failure. Major recent advances in our understanding of the molecular basis of haematopoietic malignancies have allowed for far greater risk stratification using cytogenetic and next-generation sequencing (NGS) panels to identify high-risk disease. For example, the presence of the Philadelphia chromosome in acute lymphoblastic leukaemia (ALL) or the presence of high-risk cytogenetics (e.g. monosomy 7) or molecular abnormalities (FLT-3 mutation) in acute myeloid leukaemia (AML) is associated with a high risk of relapse with chemotherapy alone [4]. Prognostic scoring systems (e.g. IPSS-R for myelodysplastic syndrome, MDS) can also be used to help select patients who may benefit from transplantation [5]. Current indications for HCT are regularly reviewed and updated by the British Society for Blood and Marrow Transplantation (BSBMT) and are summarised in Table 44.1.

Table 44.1 Classification of indications for blood and marrow transplants.

Degree of consensus	Allogeneic HCT	Autologous HCT
Very high level of agreement	Poor/intermediate-risk AML CR1 AML other than CR1 Adults with ALL CR1 (sibling donor) ALL other than CR1 CML in CP1 if TKI intolerant/ refractory, T315I mutation CML in CP2, accelerated phase High-risk myelodysplasia High-risk myelofibrosis Severe aplastic anaemia	Multiple myeloma first response Relapsed Hodgkin disease Relapsed aggressive non-Hodgkin lymphoma Poor-risk neuroblastoma Germ cell tumour CR > 1
Some variation in practice between BMT units/nations	Adults with poor-risk ALL CR1 (unrelated donor) Multiple myeloma Chronic lymphocytic leukaemia Low-grade NHL Relapsed high-grade lymphoma Low-risk myelodysplasia Low/intermediate-risk myelofibrosis Relapsed Hodgkin disease	Multiple myeloma (second autograft) Amyloid/POEMS Ewing's sarcoma Soft tissue sarcoma Autoimmune disease
Little evidence in support of transplant	CML in blast crisis Refractory ALL	CML AML ALL Myelodysplasia Myelofibrosis Chronic lymphocytic leukaemia

ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; BMT, bone marrow transplant; CML, chronic myeloid leukaemia; CP, chronic phase of CML; CR, complete remission; CR1, first complete remission; HCT, haematopoietic cell transplant; NHL, non-Hodgkin lymphoma; POEMS, polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes; TKI, tyrosine kinase inhibitor.
Source: https://bsbmtct.org/indications-table

Source of Haematopoietic Cells

Autologous Cells

Autologous cells are collected from the patient and cryopreserved prior to transplant. The infusion of these cells allows haematopoietic rescue, but the graft does not demonstrate a GvT effect. Intriguingly, autologous grafts are thought to have an immunomodulatory effect, explaining their utility in the inflammatory stages of autoimmune conditions, although the basis of this effect(s) is not yet fully understood.

Syngeneic Cells

Syngeneic cells are collected from an identical twin and with similar attributes to autologous cells.

Allogeneic Cells

The preferred allogeneic donor is a healthy human leucocyte antigen (HLA)-matched sibling, but one is only available in around 30% of patients requiring an HSC transplant. Improved testing of unrelated donor and recipient HLA genes (Class I [HLA-A, -B, -C] and Class II [HLA-DRB1, -DQB1, -DPB1]) using ultra-high-resolution NGS typing has improved transplant outcomes when using HLA-matched (12/12) unrelated donors from volunteer donor registries. However, despite increasing donor registry size and international cooperation, a fully matched donor is not always available. This is particularly true for patients of black, Asian and minority ethnic (BAME) groups and the international BMT community is working hard to achieve equality of access to donors for all. If a fully matched donor is not available, alternative options include single HLA-antigen mismatched (9/10) unrelated donors, cord blood or haploidentical related donors, with the toxicity and outcomes of these procedures steadily improving (Table 44.2) [6–8].

- *Cord blood*: umbilical cord blood from unrelated donor cord blood banks was initially used for individuals with low bodyweight (<50 kg) because of the small number of haematopoietic cells available. However, in adults and larger children, using two different cord blood units improves engraftment and is now standard of care [7]. Although two cord blood units are infused, only one unit will prevail to provide long-term engraftment. The advantage of cord blood is that it is obtained from an immune naive source with greater capacity for immunological tolerance, so that HLA and other mismatches are better tolerated with less GvHD, allowing the selection of donor cords matched just to HLA-A, -B, -C and -DR (8/8). This source of donor cells is of particular importance to BAME patients, where finding an unrelated donor can be a particular challenge,
- *Haploidentical donors*: initial results with haploidentical donors were poor due to the high level of immune suppression and T-cell depletion required to prevent life-threatening GvHD, causing high rates of relapse. However, with the development of new conditioning regimens using post-transplant cyclophosphamide, GvHD can be reduced without the high rates of relapse [8]. The use of haploidentical donors is now increasing, since most patients will have a haploidentical donor available (i.e. sibling, parent or child).

Donor Care and Selection

When more than one donor is available, other factors in addition to HLA matching should be considered, including cytomegalovirus (CMV) immunoglobulin (Ig)G serostatus of donor and recipient, donor age, sex and blood group. CMV matching between donor and recipient is important to minimise the risk of CMV reactivation post transplant, although the importance of this may change in the next few years following the introduction of letermovir prophylaxis for CMV-positive recipients. Young male donors are generally preferred, as multiparous female

Table 44.2 Comparison of sources of haematopoietic cells.

	Sibling	Family haploidentical donor	Unrelated adult volunteer	Umbilical cord blood
Availability	~30% of patients have a sibling donor match (25% chance of any one sibling being matched)	Almost every patient will have a donor (sibling/parent/child)	> 38 million donors worldwide; about 70% chance of finding a matched donor for those of Western European origin	800 000 banked worldwide; 99% chance of finding a 4/6 HLA-A, -B or -DR match
Matching requirements	Increasingly molecular matching, with 9/10 allele match acceptable	5/10	High-resolution molecular matching; 10/10 (HLA-A, -B, -C, -DR, -DQ); 9/10 allele match acceptable	Traditionally: Low-resolution (serological) for class 1 (HLA-A and -B) and high-resolution (molecular) for class 2 (HLA-DR); 6/6 preferred but can use 4/6 (subject to sufficient cell dose) Current recommendations: High-resolution molecular matching; 8/8 (HLA-A, -B, -C, -DRB1); 8/8 preferred but can use 5–7/8 (subject to sufficient cell dose)
Speed of availability	3–4 weeks, can be quicker	As per sibling	3–4 months, can be quicker but difficult	Potentially available in days from identifying the preferred cord blood(s)
Engraftment	PBSC ~14 days; BM ~21 days	As for sibling, though higher risk of rejection	As for sibling	~20–30 days; platelets may be slower in adult-size recipients
Acute GvHD (grade II–IV)	25–50% (highest with multiparous female donors)	20–40%, though may be severe	30–70%	30–70%
Chronic GvHD	30–40% for BM; 40–70% for PBSC (highest with multiparous female donors)	10–20%	40–50% for BM; 50–70% for PBSC	20–40%
Second donations/DLI availability	Availability dependent on donor	Yes, but high risk of GvHD	Availability dependent on donor	Unavailable
Risk to the donor	Small	Small	Small	None
Pretransplant testing complete (HLA and virology)	Once donor identified, takes a week or so	As per sibling	Once donor identified and requested, may take several weeks	At time of cryopreservation and unit available for issue

BM, bone marrow; DLI, donor lymphocyte infusion; GvHD, graft-versus-host disease; HLA, human leucocyte antigen; PBSC, peripheral blood stem cell.

donors can increase the risk of chronic GvHD and usually have lower bodyweight [8]. If there is an HLA mismatch between recipient and donor, the recipient should also be screened for anti-HLA antibodies and a donor selected whose HLA type does not match the specificity to these antibodies. An ABO-compatible donor is also preferable, although not essential. Scoring systems to aid the automated selection of donors have been created and it is anticipated that artificial intelligence may be helpful in better selecting donors in the future, given the large amount of registry data available for their training, but this is not yet established in clinical practice.

Donors must always be treated with respect and the patient must not be used to transmit information to a potential sibling donor. A physician separate from the transplant team should take responsibility for donor care. The Human Tissue Authority (HTA) and the Joint Accreditation Committee of the European Group for Blood and Marrow Transplantation (EBMT) and the International Society for Cytotherapy-Europe (JACIE) have recommendations regarding donor care and, where the donor is a child, an independent assessor is essential. Doctors involved in advising donors, whether family or unrelated, must be aware of current guidance and legislation [9].

Collecting Haematopoietic Cells

Haematopoietic cells may be obtained directly from the bone marrow or by peripheral blood stem cell (PBSC) harvesting. Bone marrow harvesting involves direct aspiration from both posterior iliac crests under general anaesthesia (target total nucleated cell dose of $2–4 \times 10^8$/kg) and usually requires overnight hospital admission. PBSC mobilisation, now more commonly used, involves collecting stem cells using cytapheresis over one or two days as an outpatient (target CD34+ dose of 4×10^6/kg). In healthy donors, PBSCs are mobilised using growth factor (granulocyte-colony stimulating factor, G-CSF) injections, while patients undergoing autologous procedures receive G-CSF alone or a combination of chemotherapy and G-CSF.

The choice of whether to use BM or PBSC is dependent on donor preference, although some donors may be unfit for one method or the other. PBSC allografts produce more chronic, but not acute, GvHD than bone marrow transplants in siblings. This may be associated with less relapse in patients at high risk of recurrent disease [10]. Comparative data in unrelated donor transplants also suggest that the use of PBSC is associated with more chronic GvHD, but no difference in long-term survival [11]. As a result, BM harvests are usually preferred for patients being treated for non-malignant conditions (e.g. aplastic anaemia), when a GvT response is less important, to minimise the risk of GvHD.

Complications of Transplantation

Patients who are being considered for any form of HCT must be given full information about the procedure prior to giving consent. All HCT procedures carry major risks of mortality, morbidity and long-term complications, and careful assessment of all potential transplant candidates is mandatory [12].

Regimen-Related Toxicity

The radiotherapy and chemotherapy conditioning regimen used in HCT can cause significant toxicity, including mucositis, gut toxicity and reversible alopecia. Less commonly, the liver, heart, lungs and kidneys may suffer transient or even permanent damage, and careful pretransplant assessment of the major organ function (heart, lungs, liver and kidneys) is essential.

For older patients (> 40 years) and/or those with significant co-morbidities, reduced-intensity conditioning (RIC) transplants have been developed to harness the immunological

benefits of allogeneic transplants, while avoiding much of the acute toxicity associated with the conditioning regimen [13]. RIC cytotoxic therapy is insufficient to completely ablate the recipient's bone marrow cells. Instead, RIC allografts rely on the immune-mediated effects of immune tolerance to facilitate engraftment of the donor transplant and the subsequent GvT effect to eradicate the underlying disease. DLI may also be used after the transplant to convert mixed-donor chimerism to full chimerism and treat early signs of disease relapse. The prophylactic use of DLI at six months to reduce early posttransplant relapse is currently being investigated in the proDLI trial (NCT02856464), which is expected to report its results in the near future. Although RIC transplants are sometimes known as 'mini-transplants', they are still intensive procedures requiring great commitment from the patient. Use of validated pretransplant co-morbidity scoring systems (e.g. the Sorror co-morbidity index) can estimate the transplant-related mortality (TRM) risk for individual patients and may aid in decisions [14].

Rejection

Rejection is an immune-mediated event in which the pretransplant conditioning and immunosuppression are insufficient to prevent residual recipient immune cells rejecting the donor cells. It only occurs in allogeneic transplants, although graft failure due to inadequate numbers of HSC in the transplant and/or pre-existing damage to the marrow microenvironment can occur in autologous transplants. HLA incompatibility between patient and donor, prior sensitisation of the patient to HLA or other cell antigens, T-cell depletion of the graft and low cell count are risk factors for rejection.

Graft-versus-Host Disease

GvHD is the clinical manifestation of the alloimmune response seen when immune-competent donor T lymphocytes recognise recipient antigens as foreign. GvHD is stimulated by tissue damage caused by conditioning therapy producing a pro-inflammatory environment, in which clonal donor T-cell expansion and cytokine release are promoted [15]. The risk of GvHD is increased by HLA mismatches between donor and recipient and use of unrelated donors. Despite prophylactic immunosuppression, many of the patients receiving allogeneic transplants will develop acute GvHD within the first 100 days. Acute GvHD is characterised histologically by apoptosis of epithelial cells with infiltrating immune cells. Clinically, there is a spectrum of symptoms affecting three organs:

- *Skin*: an erythematous sunburn-like rash that can progress to a blistering, exfoliative erythroderma.
- *Liver*: typically involves the bile ducts with features of obstructive jaundice, although a hepatitic variant may lead to isolated elevated transaminases.
- *Gastrointestinal tract*: lower gut involvement is characterised by profuse watery diarrhoea, bloody and accompanied by abdominal pain or ileus in the most severe cases. Upper gastrointestinal upset is not uncommon, with anorexia, nausea and vomiting.

Microbial drivers of Graft-versus-Host Disease

Acute GvHD is initiated by interaction between antigen-presenting cells (APCs) of the recipient priming allogeneic donor T cells transferred from the graft. Primed T cells differentiate into pro-inflammatory Th1 and Th17 effector cells, with subsequent damage to recipient tissue. APCs resident in the gastrointestinal tract, activated by signals derived from damaged microbial components from the microbiota, represent a major site for pathogenic T-cell induction. Indeed, the gut is the most frequently affected body site, with pathology here a major determinant of mortality. Many studies are exploring which microbial components in the gut activate donor T cells, and how this in turn drives acute GvHD (aGvHD). An ongoing challenge to successful BMT is separating the unwanted aGvHD to the desired GvT response. Understanding the local immune pathways in the gut that induce aGVHD will help to

identify interactions that separate it from the systemic GvT effect.

The human gut is colonised by a large and diverse number of commensal bacteria, and the immune system here has evolved to coexist with the microbiota in a symbiotic manner. Maladaptation of this host–microbial crosstalk can lead to inflammatory responses, with dysbiosis emerging as a major determinant of aGvHD [16–18]. Studies have largely focused on clinical outcome, and there is currently poor understanding of how commensal microbiota influence the gut immune response in the setting of BMT to drive GvHD disease. A link between microbiota and outcome has been observed in patients receiving T-cell-replete transplant grafts, suggesting that microbial-responsive donor T cells from the transplant graft could be a major driver of the GvHD and GvT response [16,19].

So, the intestinal microbiome plays a pivotal role in gut-associated aGVHD pathology. How distinct bacterial communities in the gut modulate the differentiation of donor T cells in an antigen-specific manner to drive inflammation is presently unknown. Furthermore, whether such microbe-reactive donor T cells primed in the gut can subsequently impact the GvT response away from the gut is unclear.

Chronic GvHD usually occurs later and has been reported in up to 70% of allogeneic HCTs, depending on the type of donor and conditioning/immunotherapy used. Chronic GvHD can involve any organ of the body with typical features of autoimmune disease, including dry eyes, scleroderma, hypo/hyperpigmentation of skin, lichen planus of the mouth, bronchiolitis obliterans and fasciitis/myositis [20].

Treatment strategies for reducing the risk of developing GvHD have focused on suppression of T-cell activation using systemic calcineurin inhibitors or cyclosporin, or more recently through T-cell depletion of the graft by using posttransplant cyclophosphamide. The latter has transformed the outcomes of patients having haploidentical transplantation [8] and its use in mismatched unrelated donors is an area of active research, with early phase 2 data being very encouraging.

If GvHD develops, then immunosuppression with topical or systemic corticosteroid +/- ciclosporin can be sufficient to induce immune tolerance. However, if it is steroid refractory, the UK National Institute for Health and Care Excellence (NICE) has recently approved the use of the JAK inhibitor ruxolitinib and extracorporeal photopheresis in both acute and chronic GvHD settings [21].

Relapse

Despite the intensive preparation for transplant, a significant proportion of patients will suffer recurrent disease post transplant, and this remains the major cause of transplant failure in HCT for malignant disease. Patients most at risk are those not in remission at the time of transplant or patients with more advanced disease, i.e. already relapsed after chemotherapy. Absence of GvT effect, as in autologous HCT or when no GvHD is seen, also increases relapse risk, as does the use of RIC allografts [2].

Infectious Complications

In allogeneic HCT, the conditioning regimen causes profound immune suppression to prevent rejection of the graft and the myelotoxicity results in neutropenia. In addition, immunosuppression is given for the first few months after transplant to control the new donor-derived immune response and reduce the incidence of GvHD. While haematopoietic recovery usually occurs within 2–3 weeks, full immune reconstitution can take much longer (12–18 months), making the recipient vulnerable to infections. Immune deficiency is further compounded by the presence of active GvHD and/or continued immunotherapy.

HCT-related immune problems may be divided into three phases.

Immediately Post Haematopoietic Cell Transplantation

Problems in this phase are characterised by severe neutropenia, lymphopenia and hypogammaglobulinaemia. During this period the patient is managed with the following:

- Protective isolation, clean diet and filtered air to reduce fungal infections.

- Routine prophylactic azole antifungals to prevent invasive aspergillus infection (e.g. posaconazole), antivirals to prevent CMV (e.g. letermovir) or varicella zoster virus (VZV; e.g. aciclovir), reactivation and antibacterial therapy to prevent capsulated bacterial infection (e.g. phenoxymethylpenicillin V).
- Pre-emptive use of therapeutic antimicrobials, including broad-spectrum antibiotics at the first sign of fever (temperature > 38 °C), followed by antifungal treatment in the absence of prompt resolution.

Early Post Engraftment

Following neutrophil recovery, the patient will now have marrow function and may be able to leave hospital. Although autologous transplant recipients rarely have major problems after this time, vigilance is necessary. In contrast, allogeneic HCT recipients remain at risk of the following:

- Bacterial infections related to central lines.
- Fungal infection.
- Viral infections. Most units will monitor for CMV reactivation using a polymerase chain reaction (PCR)-based test and treat patients with positive results before there is evidence of disease. Pre-emptive strategies are very effective and CMV is becoming a less important cause of mortality after allogeneic HCT [22]. Such monitoring may be applied to other viruses (Epstein–Barr virus, adenovirus) and patients are clinically monitored for respiratory viruses and herpes viruses, e.g. herpes zoster.
- Toxoplasmosis and pneumocystis.

Late Problems

Patients who have active GvHD requiring immunosuppressive therapy will continue to have impaired immunity, and most patients who have received unrelated donor transplants will have detectable abnormalities of the immune system. However, by three years post transplant, most patients off immunosuppressive drugs will have almost normal immunity. Nevertheless, there are important points to consider:

- Revaccination against common pathogens (e.g. polio, tetanus, measles/mumps/rubella [MMR]) is required, although patients still on immunosuppression may not respond optimally. HCT patients should not receive live vaccines (other than MMR). [23]
- Allogeneic transplant recipients are functionally hyposplenic and should be vaccinated against *Pneumococcus*, *Meningococcus* and *Haemophilus influenzae B* (HIB), as well as receiving lifelong prophylaxis, e.g. penicillin V.

Late Effects

There is a growing population of HCT survivors; 85% of patients alive two years after transplant will go on to become long-term survivors [24]. However, there are many other medical problems that can occur in the years following HCT, including:

- Cataracts.
- Endocrine disorders such as hypothyroidism, growth retardation in children (especially after total body irradiation [TBI] +/– steroids), metabolic syndrome.
- Sexual dysfunction and infertility.
- Second malignancies.
- Iron overload and liver dysfunction from red cell transfusions.

Haematopoietic cell transplant recipients therefore require long follow-up at a centre familiar with the range of late complications and with a sufficiently large practice to ensure that emerging problems are identified promptly. There are published recommendations for the follow-up of these patients [25].

Bone Marrow Transplant Outcome

A detailed discussion of the results of HCT is beyond the scope of this chapter as the results are dependent upon many factors, including diagnosis (e.g. AML, ALL); remission status at transplant (first complete remission [CR1], second complete remission [CR2], partial remission [PR]); transplant type (autologous, allogeneic); donor type

(sibling, unrelated); HLA matching (matched, mismatched); recipient age; and co-morbidities. Current data and results from the EBMT and the Center for International Blood and Marrow Transplant Research (CIBMTR) are available on their websites and provide up-to-date, precise information.

Registry data are of great importance, but cannot replace the careful assessment of individual patients in the light of their specific prognostic factors, such as co-existent disease, toxic effects of prior chemotherapy or previous invasive fungal infection. Also, registries report only data from patients with a minimum of three years' follow-up, and so evaluation of more recent developments requires scrutiny of primary research publications and reports to specialist meetings.

Post–Bone Marrow Transplant Chimerism and Molecular Monitoring

It has been possible to monitor leukaemic clones using sensitive molecular techniques for nearly 20 years. More recently, molecular techniques have been applied to routine monitoring of donor and recipient chimerism post transplant. RIC transplants often exhibit a period of mixed chimerism early post transplant, when the presence of both residual host and donor haematopoiesis is detectable. Mixed chimerism is associated with a higher risk of graft rejection or relapse and optimal GvT responses are dependent upon full-donor chimerism [26]. Donor lymphocyte infusions (DLI) of graded numbers of T lymphocytes can be used to drive haematopoiesis from mixed- to full-donor chimerism. However, this carries a significant risk of GvHD, as the particular subset of T cells that will generate GvT without GvHD has yet to be identified.

New techniques to detect measurable residual disease (MRD) using multiparameter flow cytometry and molecular PCR-based methods are now commonly used in many haematological malignancies to guide treatment in addition to traditional morphological-based assessments. Given the role of MRD monitoring after transplantation, however, it is likely that early detection of relapse will transform posttransplant care in the coming years.

Regulatory Aspects of Haematopoietic Cell Transplantation

An awareness of current regulations regarding HCT is essential for medical, nursing and scientific staff responsible for HCT services. In the European Union (EU), competent authorities regulate tissue banks, which include processing and storage of HSC. In the UK, for example, the HTA is the competent authority to ensure that the EU Directive on Tissues and Cells is implemented and it has legal powers. A professional international organisation, JACIE, inspects and sets standards for the safety and quality management of clinical and laboratory HCT processes. Although not having legal force, JACIE compliance is seen as vital to a safe and active HCT programme.

Conclusion and Future Perspectives

In many cases, haematopoietic cell transplants can save the lives of patients with incurable leukaemia and lymphomas. Patients who survive the first five years are likely to enjoy long-term survival, although life expectancy does not return to normal. RIC transplants have extended the benefits to more patients who might have been unfit to undergo the rigours of a myeloablative procedure and now make up around 40% of all allogeneic HCTs. Over the last 30 years the number of patients undergoing HCT has increased 10-fold and continues to rise year on year. With greater numbers of patients requiring treatment, emphasis has shifted to try to keep patients out of hospital for as long as possible through the introduction of ambulatory conditioning regimes. This has been well received by patients, as it is well recognised that receiving a transplant is a

major psychological as well as a physiological undertaking. Patient groups are increasingly being consulted when planning new clinical trials and patient-related outcomes are routinely becoming incorporated in their objectives. Development of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) in the USA and more recently the Improving the Management of symPtoms during And following Cancer Treatment (IMPACT) consortium in the UK has provided a framework within which to develop new clinical trials, resulting in a resurgence of trials in the field. The recent advances in prophylactic antivirals and antifungals and therapies for treating GvHD are making HCT safer than ever before. However, with the emergence of new, less intensive cellular therapies such as chimeric antigen receptor T-cell therapy (CAR-T), it is unclear what role HCT will fulfil in the future; potentially it will be reserved for patients failing cellular therapy or those with non-malignant disease.

KEY POINTS

1) Haematopoietic stem cell transplants provide a curative treatment for patients with haematological malignancies, but are associated with significant risks of morbidity and mortality.
2) The number of transplants (autologous and allogeneic) performed each year is increasing worldwide, although the indications, type of transplant and donor source have changed over time.
3) Although reduced-intensity conditioning transplants have less immediate toxicity than conventional myeloablative transplants, they remain arduous procedures, with many short- and medium-term complications.
4) National and international volunteer unrelated donor registries continue to expand, with particular emphasis on recruiting young, healthy, male donors and donors from black, Asian and minority ethnic groups.
5) Use of haploidentical donors has increased in recent years, with the corresponding use of cord blood units declining.
6) Outcomes are continuing to improve thanks to improved donor selection using ultra-high-resolution human leucocyte antigen typing, reduced graft-versus-host disease due to posttransplant cyclophosphamide and improvements in prophylactic antimicrobials.
7) Randomised controlled clinical trials remain the best way to produce definitive data and the creation of the IMPACT and BMT CTN networks has provided a framework to support clinical trial development that will continue to improve the safety and efficacy of haematopoietic cell transplantation.

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45 CAR-T Cells and Recent Advances in Clinical Cellular Immunotherapy

Yisu Gu[1,2,3], Alex Rampotas[1], Connor Sweeney[1,2], David J. Roberts[1,2,4] and Ronjon Chakraverty[1,2]

[1] *Department of Haematology, Churchill Hospital, Oxford, UK*
[2] *BRC Haematology Theme, Radcliffe Department of Medicine, University of Oxford, UK*
[3] *Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK*
[4] *NHS Blood and Transplant, Oxford, UK*

Ἐς δὲ τα ἔσχατα νουσήματα αἰἔσχαται θεραπεῖαι ἐς ἀκριβείην, κράτισται.
Extreme therapies are indicated for the worst kind of diseases
— Hippocrates, Aphorisms Section 1;6

The infusion of donor-derived T cells (donor lymphocyte infusion, DLI) is now common practice following allogeneic haemopoietic stem cell transplantation (HSCT) to prevent or treat relapse of blood cancer, but only in the last decade have advances in genetic engineering and cell manufacture permitted the use of redirected patient-derived (autologous) T cells towards specific cancer or viral antigens. Cellular immunotherapy is now the focus of huge attention and investment from the biopharmaceutical industry, with many early-phase trials demonstrating impressive clinical responses.

Cellular Immunotherapy in Haemopoietic Progenitor Cell Transplantation

The anti-leukaemic activity of allogeneic bone marrow transplantation was first described in murine experiments more than 60 years ago, but was appreciated in the clinic only in the late 1970s when attempts at preventing graft-versus-host disease (GvHD) by T-cell depletion were sometimes frustrated by an increase in the risk of leukaemia recurrence. The clinical anti-leukaemic effect of GvHD was first reported in 1979 and confirmed later by registry data from the International Bone Marrow Transplant Registry (IBMTR) [1]. The observed benefit of GvHD was particularly evident in patients transplanted for chronic myeloid leukaemia and led to the widespread use of post-transplant DLI, where lymphocytes from the original donor were infused into patients following disease relapse. The first peer-reviewed report of DLI therapy included a single patient who achieved molecular remission with no evidence of clinical GvHD, supporting the hypothesis that graft-versus-leukaemia (GvL) could be directed at leukaemia-restricted target antigens or recipient haematopoiesis [2]. T cells contained within the donor lymphocytes induce GvL through recognition of histocompatibility antigens, tumour-associated or tumour-specific antigens.

Currently, many allogeneic HSCT regimens incorporating T-cell depletion at the time of transplant (for the prevention of

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GvHD) include posttransplant DLI to boost immune reconstitution, or to treat mixed chimerism or disease relapse/progression. The risk of GvHD after DLI has been somewhat reduced by the use of incremental doses of DLI given several weeks apart, but the search for the 'holy grail' of leukaemia-specific GvL while limiting GvHD continues to be an active research theme.

Non-specific T-Cell Immunotherapy

In the autologous setting, there is clear evidence that T-cell immunity can not only eradicate infections but can also protect against cancer. Thus, patients with inherited or treatment-related defects in T-cell immunity are at higher risk of cancer development. On the other hand, biologics that enhance endogenous T-cell functions (bi-specific T-cell engagers or immune checkpoint inhibitors, blocking inhibitory pathways such as CTLA-4 and PD-1) have shown impressive clinical benefits in cancer patients who had failed all conventional therapy options [3–6].

In the allogeneic setting, dissecting GvHD from GvL has been attempted by the removal of alloreactive T cells from donor grafts, while retaining non-alloreactive cells that could mediate GvL and antiviral responses. In some trials, clinical-scale *ex vivo* stimulation of allogeneic donor T cells with normal haemopoietic cells from the recipient is used to stimulate a mixed lymphocyte response. Reactive T cells, identified by the expression of activation antigens (e.g. CD25, CD69), are then depleted by immunotoxin or immunomagnetic selection. Although this and similar approaches may permit preservation of antiviral immune responses, the extent to which GvL is preserved is unknown. The expense of this approach and technical issues, for example the generation of random oligoclonal responses and lack of day-to-day reproducibility, may limit the widespread adoption of this approach.

Another strategy has been the selective depletion of CD8+ T cells from DLI [7]. This approach is based upon the rationale that infusions of products enriched for allogeneic CD4+ T cells will be likely to induce less GvHD because peripheral tissues mostly lack expression of human leucocyte antigen (HLA) class II, whereas blood cancers usually express HLA class II antigens and can be targeted. Evidence of GvL, resolution of mixed T-cell chimerism and improved antiviral immunity in the absence of GvHD have all been reported in clinical trials of CD8-depleted DLI. Trials of this form of immunotherapy are continuing and are reporting encouraging results with respect to reversal of mixed chimerism and GvL [8].

While these studies show proof of concept that adoptive transfer of T cells can be exploited therapeutically, greater precision will rely upon a better understanding of the antigens recognised by T cells and how they interact with the complex immune landscape of cancer.

The Immune Landscape of Cancer

The tumour microenvironment (TME) refers to the local area immediately surrounding tumour cells; it is composed of a heterogeneous population of resident and recruited host cells, including innate and adaptive immune cells, signalling molecules and extracellular matrix proteins, and represents the site for complex interactions between the immune system and tumour. Through activation of the innate and adaptive immune system, developing tumours potentially are subject to three phases of interaction with the host: elimination, equilibrium and escape (sometimes referred to as the '3 Es'). In the first phase, a robust anti-tumour immune response eliminates malignant cells; if this process eliminates all malignant cells, no further tumour growth can occur. However, if a small number of tumour subclones survive,

immune surveillance may be sufficient to hold the tumours temporarily in check. The selective pressure of the immune system may then eventually select for tumours capable of evading immunosurveillance, leading to escape and tumour progression.

Understanding the crosstalk between tumour cells and the immune microenvironment during each phase of cancer progression underpins the development of more personalised immunotherapies. Historically, immunohistochemistry has been used to study the spatial composition of tumours and their normal adjacent tissue. However, these methods can detect only a limited number of surface and intracellular proteins. Single-cell technologies (such as multiparameter flow or mass cytometry, or single-cell RNA sequencing) have now revealed unprecedented levels of heterogeneity within tumour and immune cell populations. This new information has shown, for example, why certain immunotherapies fail, by defining molecular signatures associated with T-cell exhaustion and anergy. More recently, several spatial-omics technology platforms allow profiling of not only cell function (with transcriptomic, proteomic and metabolomic readouts), but also structural composition of tumour tissue sections. Spatial-omics demonstrate some causes of poor immunotherapy response, such as infiltration of only exhausted T cells into the tumour niche during checkpoint blockade therapy, or endogenous cytotoxic T cells and/or chimeric antigen receptor (CAR)-T cells remaining excluded from the tumour by physical barriers, such as trapping by stroma [9]. Furthermore, highly organised ectopic lymphoid structures, known as tertiary lymphoid structures (TLS), have been found in tumour tissues that participate in the response to checkpoint blockade therapy, with B cells within TLS associated with a more favourable prognosis [10]. These exciting studies may pave the way for new combinations of targeted immunotherapies, for example modulating the B-cell response within TLS concurrently with T cell–based therapies.

Tumour Antigens

All nucleated cells in the body, including cancer cells, present self-peptides on their surface bound to HLA. This enables immune surveillance by T cells to detect abnormal cancerous cells, leading to their elimination. These peptide antigens on the cancer cell surface represent potential targets for immunotherapy. Ideal antigens are those that are (1) tumour specific; (2) expressed on most of the cancer cells; (3) capable of stimulating a robust T-cell response; and (4) important for the cancer phenotype, as this will prevent downregulation of expression leading to immune evasion.

There are two main categories of tumour antigens and each type has been targeted by immunotherapy in clinical trials:

- Neoantigens are tumour specific and mainly result from gene mutations or chromosomal fusions in tumour cells, which generate aberrant proteins. However, they may also be produced by dysregulation of splicing and retention of introns in RNA transcripts. Resultant tumour-specific peptides may be bound by HLA, presented at the cell surface and elicit T-cell responses. As these genetic events are acquired during carcinogenesis, they are cancer specific and therefore targeting with immunotherapy is expected to avoid toxicity to healthy tissues.
- Tumour-associated antigens (TAAs) are expressed at elevated levels in tumour cells, but are also expressed in normal cells. Many TAAs are usually only expressed in immune sanctuary sites such as the testes, or at specific stages of development (e.g. the placenta). A subset of these, the cancer-testis antigens, usually have expression restricted to male germ cells, but in cancer they may be re-expressed.

Most known tumour antigens in humans are TAAs. A low level of antigen expression in normal tissues can lead to the inactivation of T cells with high avidity receptors by immunological tolerance mechanisms. Consequently, the antigen-specific T cells that escape immune tolerance often generate

low-avidity responses that may be inadequate in providing tumour protection. In contrast, there is no pre-existing immunological self-tolerance to tumour-specific neoantigens and therefore T-cell receptors typically have higher avidity for their target. Coupled with the lower likelihood of damage to healthy tissues, neoantigens are therefore theoretically more attractive target antigens for immunotherapy by vaccination or adoptive T-cell transfer. It should be noted, however, that only a minority of genetic mutations will give rise to neoepitopes capable of provoking an immune response, and identifying such neoantigens is therefore highly resource intensive and technically challenging.

Targeting Tumour Antigens with Immunotherapy

Tumour antigens can be therapeutically targeted by vaccination or by adoptive transfer of antigen-specific T cells. Vaccination with peptide antigen aims to stimulate proliferation of the patient's own antigen-specific T-cell clones. These T cells then target cancer cells that also present the peptide, leading to immune control of disease. Most vaccination trials have been directed against melanoma antigens, but sustained clinical benefit is rare. Wilms' tumour 1 (WT1) is a TAA that is highly expressed in many cancers, including most cases of acute myeloid leukaemia (AML; see Yang et al. 2007 in Further Reading). A polyvalent vaccine has been used in early-phase clinical trials of AML in complete remission, with functional T-cell responses demonstrated *in vitro* [11].

An alternative method of targeting antigens expressed by HLA on cancer cells is by adoptive T-cell therapy. Antigen-specific T-cell receptor (TCR) gene transfer into autologous or allogeneic T cells is efficiently achieved using retroviral or lentiviral vectors, followed by infusion of the modified T cells into the patient. Modifications to the TCR construct are commonly used to enhance cell surface expression and to reduce the chance of the introduced TCR chains mispairing with endogenous TCR α and β chains; this mispairing can potentially generate TCRs with unpredictable reactivity. Compared with CAR-T cell therapy, a disadvantage is that the TCR will only recognise antigen presented by certain HLA alleles, so clinical trials typically use TCRs that recognise antigens presented by HLA alleles that are present at high frequency in the population (e.g. HLA-A*0201). WT1 has been targeted using adoptive transfer of TCR gene-modified T cells into AML patients following allogeneic HSCT. A high-affinity WT1 antigen-specific TCR was inserted into donor CD8+ T cells, which were then infused prophylactically into 12 patients. No relapses were observed after median follow-up of 44 months [12]. Preclinical studies have also targeted neoantigens in AML by TCR transfer using lentiviral vectors. TCRs targeting neoantigens resulting from *NPM1* mutations and the *CBFB–MYH11* gene fusion demonstrated AML killing *in vitro* and in murine models [13,14] and may have clinical applicability.

Chimeric Antigen Receptor T Cells

First-generation CAR-T cells were developed in the 1990s to link antibody specificity to T-cell anti-tumour cytotoxicity. The chimeric receptor consisted of a single variable chain of an antibody bound to a CD3ζ receptor. The addition of a co-stimulatory molecule (4-1BB or CD28) was applied in second-generation CAR-T cells to improve T-cell functions and has proven highly successful in the clinic. The CAR construct contained in a replication-incompetent retrovirus or lentivirus is transduced into a T cell *ex vivo*. Following use of lympho-depleting chemotherapy, the CAR-T cells are reinfused into the patient, where they have the potential to induce robust T cell–mediated anti-tumour responses and persist to provide long-term immunosurveillance (Figure 45.1).

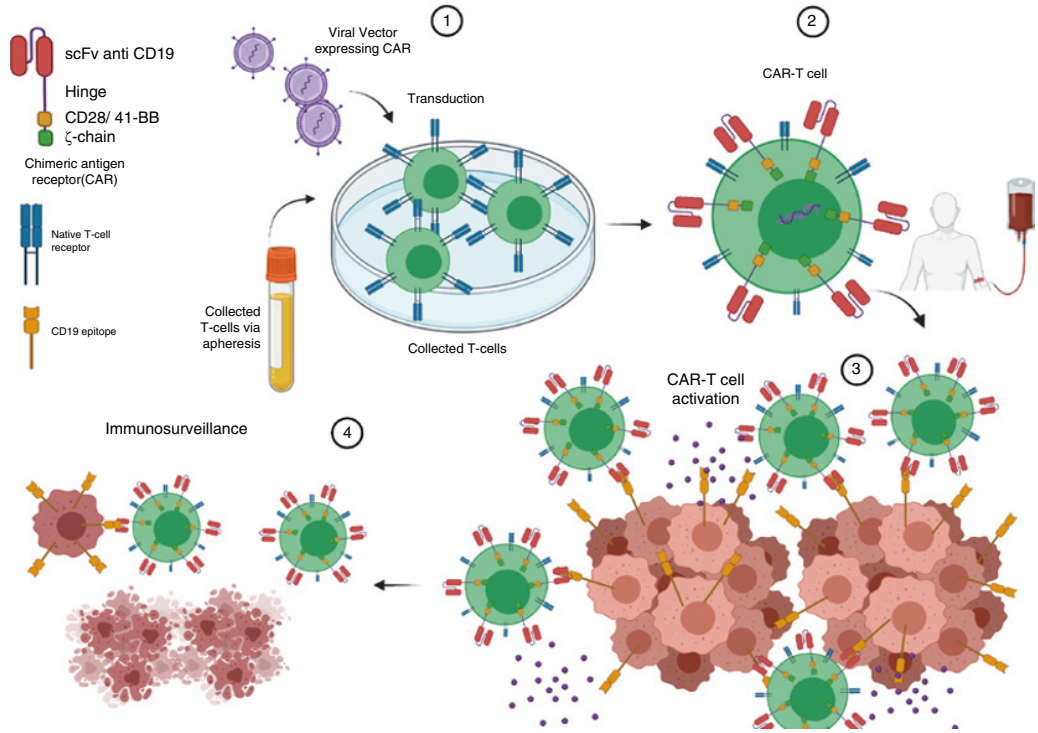

Figure 45.1 (1) T cells are collected via apheresis and are transduced with a retrovirus or lentivirus containing the chimeric antigen receptor T cell (CAR-T)-expressing gene. (2) Once CAR expression is confirmed, the cells are infused back into the patient. (3) They use antibody specificity to identify the tumour. They become activated and eliminate the tumour cells. (4) They remain in the body as a 'living drug' providing constant immunosurveillance. *Source:* made in © BioRender - biorender.com.

In phase I clinical trials, anti-CD19 CAR-T cells achieved remission rates of 90% in heavily pretreated patients with acute lymphoblastic leukaemia (ALL). The outstanding results led to multicentre phase II trials, where their efficacy was confirmed. Anti-CD19 CAR-T induce high complete-response (CR) rates of 50% in relapsed/refractory diffuse large B-cell lymphoma [15,16]. Additional trials in mantle cell and follicular lymphomas demonstrate their efficacy across multiple CD19 malignancies [17]. These results led to their regulatory approval for the treatment for these malignancies, while more recently an anti-B cell maturation antigen (BCMA) CAR-T cell product has been approved for relapsed multiple myeloma (see Cho et al. 2018 in Further Reading).

During CAR-T cell use in the trials some notable severe toxicities were reported, namely cytokine release syndrome (CRS) and immune effector cells associated neurotoxicity syndrome (ICANS). CRS (manifest by hypoxia, hypotension and end-organ dysfunction) is a pro-inflammatory response induce by CAR-T cells in concert with other immune cells such as macrophages. The precise pathophysiology of ICANS is less understood, but may relate to disturbances in the blood–brain barrier causing tremor, focal neurological deficit, seizures and encephalopathy. CRS is treated with supportive care and antibodies that inhibit the actions of interleukin (IL)-6, sometimes with the addition of steroids. ICANS is treated by supportive care, steroids and the use of anti-epilepsy medications. Prolonged cytopenias and hypogammaglobulinaemia, the latter through targeting of normal B cells, are more predictable but still significant side effects of anti-CD19 CAR-T cells.

The logistical and financial costs of CAR-T cells are a major challenge to their widespread implementation. Furthermore, the personalised nature and the expertise required to manufacture such products are costly, making it difficult for healthcare systems to rapidly incorporate these novel treatments. Additionally, long manufacturing times make many patients ineligible to receive them due to rapidly progressing disease. Allogeneic 'off-the-shelf' CAR-T cells could potentially resolve some of these challenges, but issues regarding their persistence and host rejection remain.

The ideal CAR-T cell target is an antigen that is expressed on the cell surface and can easily be recognised by the modified T cells; is expressed by the tumour cells alone (neoantigen) to avoid on-target, off-tumour toxicity; and is the product of a disease-driving mutation. These conditions for success are not met for most malignancies, which may not express suitable targets and/or are heterogeneous. Other antigens such as CD123 in AML, CD22 in B-cell acute lymphoblastic leukaemia (B-ALL) and GD2 in neuroblastoma are being explored by multiple groups. Attempts are also being made to target more than one antigen at the same time and to overcome PD-L1 or transforming growth factor (TGF)-β inhibition. More sophisticated engineering could potentially overcome these problems, but at least the current therapies have been highly successful in treating some haematological malignancies where all other treatments had poor results.

Bispecific T-Cell Engagers and Bispecific Antibodies

A more pharmacological 'off-the-shelf' approach to engage native T cells against malignant cells is by using bispecific T-cell engagers (BiTEs) and bispecific antibodies (BSAs). BSAs aim to bring the T cells in proximity to the cancer cells so that a T cell–mediated immune response is induced. BiTEs lack the Fc portion of the antibody, which necessitates a continuous infusion as their half-life is very short. BSAs make use of the Fc segment to activate immune effector cells and induce a broader immune response. Blinatumumab, an anti-CD19/CD3 BiTE, has been approved for use in relapsed B-ALL, while many BSAs have shown good initial results in early phase trials in diffuse large B-cell lymphoma (DLBCL) [18].

Tumour-Restricted Natural Killer Cell Immunotherapy

Early trials of natural killer (NK)-cell immunotherapy were based upon infusion of autologous NK cell–activating cytokines or *ex vivo* activated NK cells, but were largely disappointing. However, understanding the complex mechanisms underlying NK cell function is a prerequisite for successful NK-cell immunotherapy (Figure 45.2).

Human NK cells are controlled by inhibitory and stimulatory signals through cell surface receptors, which allow them to distinguish between normal and malignant or infected cells. All human NK cells express multiple inhibitory and activating receptors that define functional subsets of NK cells.

In the 1980s, Klaus Karre showed that murine NK cells preferentially lysed major histocompatibility complex (MHC) class I negative tumours. We now know that killer immunoglobulin-like receptors (KIRs) bind to HLA class I molecules, and the majority of KIRs transduce inhibitory signals upon ligation by their specific HLA class I ligand [19]. NK-activating signals may be provided via sequential stimulation of receptors, though their natural ligands remain largely unknown [20].

The clinical relevance of NK-cell inhibition is evident in haploidentical HSCT, where certain HLA class I mismatches generate HLA: KIR incompatibility that allows NK-cell activation. Highly significant reductions in relapse have been reported among AML patients receiving HLA: KIR–incompatible haploidentical HSC grafts, compared to patients receiving grafts in which the donor NK repertoire is matched to the HLA type of the patient.

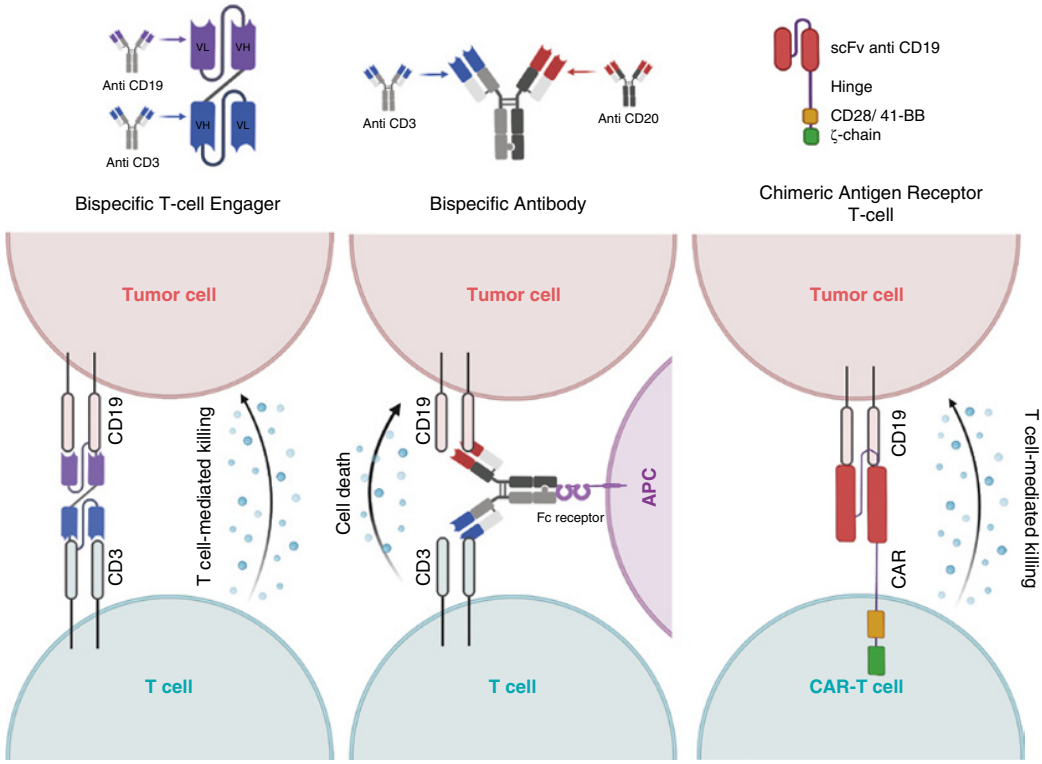

Figure 45.2 Types of T-cell immunotherapy. Bispecific T-cell engagers (BiTEs) induce a T cell–mediated killing by bringing native T cells closer to the tumour cells. Bispecific antibodies are similar to BiTEs but contain the Fc portion of the antibody, which activates immune effector cells against the tumour. Chimeric antigen receptor (CAR)-T cells are directly activated by the presence of the target antigen, as they carry a chimeric receptor that binds antibody specificity with T-cell activation. *Source:* made in © BioRender - biorender.com.

These observations encouraged the development of NK-cell immunotherapy. Allogeneic IL-2-activated NK cells from HLA-mismatched donors were used to treat AML patients. High NK cell dose and extensive preinfusion conditioning achieved complete remission with donor NK cell engraftment and normal bone marrow function, but the response was short-lived. Recent studies have tried to overcome this limitation.

The efficacy of haploidentical NK cells expanded with IL-2 may be hampered by concurrent stimulation of inhibitory regulatory T cells (Tregs). The NK homeostatic factor IL-15 has been used to stabilise NK-cell number and function. Patients with refractory AML given recombinant human IL-15 (rhIL-15) after lympho-depleting chemotherapy and haploidentical NK cells achieved better rates of *in vivo* NK-cell expansion and remission compared with previous trials with IL-2. However, subcutaneous but not intravenous IL-15 was associated with previously unreported CRS, although a route for rhIL-15 given with expanded NK cells may allow effective and durable anti-leukaemic responses with low toxicity [21,22].

Other techniques may harness the NK effector cells' function. NK cells, modified to express an anti-CD19 CAR, were effective in the treatment of patients with B-cell lymphoma and with less toxicity than usually seen with CAR-T cells [23]. NK-cell immunotherapy may yet provide effective therapy for haematological malignancies (see Gurney and O'Dwyer 2021 in Further Reading).

Passive Cellular Immunotherapy of Infectious Disease

Cellular immunotherapies have also been used in the treatment of opportunistic viral infections in immunocompromised patients. Most of these trials have been in the post-transplant setting, particularly in recipients of allo-HSCT grafts. The earliest studies involved infusion of enormous numbers of cloned cytomegalovirus (CMV)-reactive CD8 T cells, which caused resolution of refractory CMV disease in patients post allo-HSCT. Subsequently, others elegantly demonstrated the specific resolution of posttransplant Epstein–Barr virus (EBV)-driven lymphoma following infusion of donor-derived anti-EBV CTLs. *Ex vivo* generation of very large numbers of antiviral T cells is complex and expensive. However, in 2003, a phase I trial of allogeneic donor-derived CMV-reactive T cells grown for 21–28 days *ex vivo* on monocyte-derived dendritic cells, pulsed with fixed whole CMV, resolved the reactivation of CMV in 8/16 post allo-HSCT patients without recourse to antiviral chemotherapy. T-cell doses were < 10^5 T cells/kg bodyweight and the average dose of CMV-specific T cells in each dose was < 200–300 per kg. Despite this incredibly low dose of cells, virus-specific T cells were detectable in the peripheral blood of responding recipients at levels equivalent to a 35 000-fold expansion [23].

However, these early trials did not lead to the widescale adoption of pathogen-specific cellular therapy, due to the extreme technical complexity of cell manufacture. Alternative approaches include the immunomagnetic selection of specifically activated T cells based on the secretion of interferon (IFN)-γ and its capture on the cell surface with a bispecific antibody complexed to a paramagnetic nanoparticle [24]. Multimeric recombinant MHC class I complexes loaded with an immunodominant viral peptide antigen restricted to the specific class I antigen can be complexed with paramagnetic nanoparticles to select antiviral-specific CD8 T cells from donor blood [25].

Widespread adoption remains constrained by the need for directed donations (autologous or HLA-matched allogeneic), although some recent studies do support the feasibility of the ultimate goal of 'off-the-shelf' products. 'Off-the-shelf' HLA-mismatched third-party T-cell lines have been used to treat posttransplant EBV-related lymphoma in recipients of renal transplants [26], and the development of such one-to-many therapies may represent the future of adoptive cellular immunotherapy.

Why Do T-Cell Immunotherapies Fail?

Without a doubt, immune therapies have changed and continue to change the way we think about and treat cancer. However, overall efficacy remains limited, and many immunotherapies do not work for all patients. Several reasons account for this failure. First, cancer cells can secrete several key regulatory cytokines, including IL-10 and TGF. IL-10 is a well-characterised anti-inflammatory cytokine produced predominantly by macrophages, Th2 cells and Tregs. It promotes immune tolerance through several key mechanisms, including the inhibition of pro-inflammatory IL-1, IL-12 and IFN-γ produced by monocytes and macrophages, and the downregulation of co-stimulatory molecules on dendritic cells and macrophages (reviewed in [27,28]). Furthermore, cancer cells recruit several myeloid and lymphoid immune suppressor cells into the tumour microenvironment, including tumour-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and Tregs (reviewed in [29]). Secondly, tumours can cause downregulation of MHC class I on their cell surface, thereby disrupting the main antigen presentation machinery required to prime anti-tumour cytotoxic CD8+ T cells (reviewed in [30]). One strategy to overcome these mechanisms of tumour escape includes combinational therapies that target more than one immune pathway of cancer survival. For example, combining TGF inhibition with

anti-PD-L1 checkpoint blockade has shown promise in both primary breast cancer models and established liver metastasis secondary to colorectal cancer [31].

Technical Advances Facilitating Translational Research in Cellular Immunotherapy

In Europe, since the ratification of the European Union (EU) Clinical Trials Directive in Member States in 2004, most cellular immunotherapies being evaluated in trials (excluding cells that have undergone minimal manipulation *ex vivo*, e.g. DLI) have been regulated as advanced therapy investigational medicinal products (ATIMPs). Such ATIMPs must be manufactured according to good manufacturing practice (GMP) regulations. Navigating and fulfilling the regulatory requirements are complex and expensive. Use of CE-marked reagents, consumables and devices for clinical-grade cell production is one advance to help laboratories meet the regulations. Closed and semi-closed systems are available for handling large-volume cell suspensions. Gas-permeable cell culture and expansion bags allowing closed-system culture are now widely available and the availability of clinical-grade cytokines is improving.

One of the most significant advances in the field has been the development of CE-marked clinical-grade immunomagnetic cell sorters. These are now widely used for the specific selection of subsets of haematopoietic progenitor cells and other leucocytes, and can even select antigen-reactive cells on the basis of cytokine secretion, multimeric HLA peptide reagents or expression of activation markers. The CliniMACS Prodigy® machine is a good example of how a more automated process can significantly reduce the cost of CAR-T production. It has been used in academic centres by incorporating most of the production steps that would usually take place in different laboratories into a single closed-cycle machine. As the regulatory position becomes clearer, it is hoped that it will become easier for the academic and commercial sector to meet the appropriate standards, reducing costs and widening applicability.

Future Directions

Immunotherapy will clearly play a major role in the future treatment of cancer. Current products have significant limitations, but the advances in manufacturing, cell engineering and better understanding of cancer's immune evasion mechanisms will be reflected in future products. Cellular therapies will likely incorporate precision cell engineering, with CRISPR-Cas9 to allow more complex engineering such as strategies to avoid inhibition. CAR-T cells specifically will likely target more than one antigen using humanised binders and carry off-switches to prevent severe toxicities. Other cellular therapies such as CAR-NK and CAR-iNKT cells have also been found to be effective in preclinical and phase I trials. The high cost and difficulty in manufacturing will be mitigated by automation using closed and semi-closed systems. It is hoped that this will allow the widespread use of combination immunotherapies in clinical trials.

KEY POINTS

1) Allogeneic graft-versus-leukaemia by donor lymphocyte infusion is proof of principle of cellular immunotherapy.
2) Use of checkpoint inhibitors in a number of malignancies has demonstrated further the role of T cells in the control of cancer.
3) Cellular immunotherapy of viral infections is becoming an alternative to antiviral chemotherapy.
4) Gene-modified autologous and allogeneic antigen-specific T cells can be reliably generated using retroviral or lentiviral vectors.

5) Chimeric antigen receptor (CAR) and T-cell receptor (TCR)-gene-modified T cells have shown antitumour effects in several malignancies, with the most impressive clinical results observed using CD19-targeted CAR-T cells in acute lymphoblastic leukaemia and diffuse large B-cell lymphoma.
6) Technical and regulatory difficulties in the production of cell therapies are being overcome.

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46 Tissue Banking

Akila Chandrasekar, Richard Lomas and Paul Rooney

NHS Blood and Transplant, Tissue and Eye Services, Liverpool, UK

Tissue banking has become an increasingly important area of activity for EU blood services and allows expertise in donor and quality management to be applied to collect, process and store tissues in a carefully regulated environment. Consent for removal and use of all tissues must be obtained and recorded. Assessment of deceased donors must be thorough and retrieval of tissues rapid to reduce the potential for bacterial contamination and cell death/damage. Processed heart valves, tendons, ligaments, bone, skin and corneas are used in a wide variety of surgical spheres. Decellularised tissues have the advantage of not inducing an immune response and of becoming repopulated by recipient cells and eventually being remodelled by the recipient, so the allograft becomes part of the host. Further advances include using decellularised tissue as a starting material for production of human tissue hydrogels or bio-inks for 3D printing. These novel technologies herald a new era of regenerative medicine.

Regulation

The European Union Tissue and Cells Directives (EUTCD) is made up of three Directives: the parent Directive (2004/23/EC), providing the framework legislation, and two technical Directives (2006/17/EC and 2006/86/EC – amended in 2015/565), providing the detailed requirements of the EUTCD. These Directives are currently being revised following evaluation of the legislation in 2019, with planned adoption of the revisions in late 2021. In the UK, the Human Tissue Act (2004) established the Human Tissue Authority (HTA) as the competent authority, with responsibility for regulating tissues and cells (other than gametes and embryos) for human application within England, Wales and Northern Ireland. There is separate legislation in Scotland – the Human Tissue (Scotland) Act 2006 – with a high degree of similarity between both Acts. The EU Directives were fully implemented into UK law in 2007, via the Human Tissue (Quality and Safety for Human Application) Regulations 2007. Tissue Establishments (TEs) in the USA are regulated by the Food and Drug Administration (FDA).

Consent

Consent is the fundamental principle of the Human Tissue Act (2004) and underpins the lawful removal, storage and use of donated tissue for any purpose. While provisions of the Human Tissue (Scotland) Act 2006 are based on authorisation rather than consent, these are essentially both expressions of the same principle. In Europe, the legal requirements for obtaining permission for retrieval of tissues after death vary from country to country.

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However, even where 'opting out' or 'presumed consent' systems are operated, it is considered best professional practice to confirm that no relatives object to the donation proceeding.

Informed and valid consent must be obtained from an appropriate person, prior to tissue retrieval, to ensure that tissue donation for any purpose, such as clinical use or research/training, is lawful. With a living donor, the appropriate person is generally the donor themselves. For deceased donors, this can be the wishes of the deceased themselves expressed in life, for example through the organ donor register, or their nominated representative, a person who was appointed in life by the deceased to make these decisions. In the absence of either of these, the consent of a person in a 'qualifying relationship' with them immediately before they died must be sought. This may be (in order of priority) a spouse or partner, blood relation or friend.

Consent should be taken only by those trained to do so. It is important that the person giving consent is fully informed about all aspects of the donation process and, where appropriate, what the risks are. The duration of the consent must also be specified; the person giving consent may withdraw it at any point before or after donation, providing that the tissue has not already been used, and it is important that they are informed of this right. With the exception of anatomical examination or public display, where written consent is required, the Human Tissue Act does not specify the format in which consent should be recorded. Verbal consent, documented either by audio recording or in the patient's notes, is also valid.

Donor Selection and Testing

Tissue donors must be carefully selected to minimise the risk of transmitting diseases and to ensure suitable quality of grafts for transplantation. The major donor exclusion criteria described in the EU Directive (Box 46.1) are based on these two principles.

The donor selection process includes a structured interview with living donors to obtain a detailed medical and behavioural history. In the case of deceased donors, this interview is conducted with someone who knew the donor well – usually, but not always, a relative. The reliability of a family interview depends on how well the interviewee knew their deceased relative and additional sources of information can supplement the donor selection process. Information is sought from the general practitioner and, when necessary, from the referring hospital practitioner if the donor was admitted to hospital prior to death, to obtain as accurate a medical history as possible [1]. The result of postmortem examination is reviewed [1] if one was carried out.

Box 46.1 Donor exclusion criteria.

- History of disease of unknown aetiology
- Presence or past history of malignancy (some exceptions)
- Risk of transmission of prion disease(s)
- Systemic infection or significant local infection uncontrolled at the time of donation
- History or evidence of risk of transmissible viral infections such as human immunodeficiency virus (HIV) and hepatitis
- History of chronic or systemic autoimmune disease that could have a detrimental effect on the tissues
- Unknown cause of death for deceased donors

Donor blood samples for testing must be obtained at the time of donation or within seven days post donation for living donors. The sample from deceased donors must be obtained just prior to death or within 24 hours after death. Fluids administered in the 48 hours prior to death must be recorded to allow an estimation of any plasma dilution effect. Tissues from donors with plasma dilution of more than 50% can be accepted only if testing procedures used for screening are validated for such plasma dilution or if a pre-transfusion sample is available.

The minimum requirement for mandatory tests required by the EU Directive includes screening for hepatitis C (HCV: anti-HCV), hepatitis B (HBV: HBsAg and anti-HBc), human immunodeficiency virus (HIV: anti-HIV I and

II) and syphilis. Individual nations or TEs are permitted to set higher standards than the minimum requirements; for example, in the UK blood services, tissue donors are also screened for hepatitis E. There is a requirement in the EU Directive to quarantine living-tissue donations to obtain a second blood sample from the donor after an interval of 180 days to repeat the mandatory tests; however, if the blood sample taken at the time of donation is additionally tested by the nucleic acid amplification method (NAT) for HIV, HCV and HBV, a retest is not required after 180 days. In the UK blood services, all tissue donors (living and deceased) are screened by NAT for HIV, HCV and HBV, in addition to the antibody and antigen tests mentioned above. The interval between the time of infection to the onset of detectable infection on screening tests is known as the 'window period.' NAT reduces the risk of transmission of infection during the early phase of the infection following exposure to a virus, before antibodies can be detected on screening. However, the serology screen may serve as an indicator of a past exposure and as an indicator of lifestyle risks.

In addition to mandatory tests, discretionary tests may also be applied to donors based on their travel history. This applies in particular to the risk of tropical viruses. The emergence of new infectious agents should also be monitored, and donor selection and testing protocols amended accordingly. In recent times, the emergence of Sars-CoV-2 has created a particular challenge for TEs, especially in the number of donors eligible to donate tissues. A survey of European eye banks, in collaboration with the European Eye Banking Association, demonstrated a decline of up to 68% in procurement and grafting of corneas between April and June 2020 and showed different algorithms for acceptance in different European countries [2].

Tissue Procurement

Living donations are retrieved during surgery by the operating team. Clear, written instructions, staff training and standard sterile kits are provided by the TE for tissue collection. Regular auditing to ensure compliance with agreed procedures, detailed in a written agreement between the TE and the hospital, is an integral part of a living donation programme.

With deceased donors, it is important to ensure the quality of the tissues removed. Tissues can deteriorate post mortem due to microbial contamination and autolysis or be contaminated during the retrieval process. The optimal time and place to procure tissues from deceased donors are in an operating theatre, immediately after death or post cessation of circulation. However, the availability of these facilities for tissue donation is limited and is generally restricted to tissue grafts that can be obtained during routine organ procurement procedures, such as removal of the heart for valve donation. In the UK, the majority of tissue donations are performed in hospital mortuaries or on rare occasions in funeral homes.

Donor identification by means of a wristband or toe-tag is a crucial step before commencing the retrieval. A minimum of three points of identification, such as name, date of birth, hospital number and address, is required to positively identify the donor. Before tissue retrieval, a thorough external examination of the donor body appearance is conducted and recorded as part of donor assessment. This examination should include detection and recording of tattoos, jaundice, evidence of drug use, body piercing, open wounds or signs of infection, scars and bruises, intravenous cannula sites, operation incision sites and other significant abnormalities.

Reducing Bacterial Contamination

Following death, autodegradation of all tissues commences as cells die and release lytic enzymes into the tissue. The intestinal microflora begin to migrate throughout the body, contaminating other tissues. The rate of both these processes is critically dependent on temperature, so it is crucial that warm

ischaemia time is minimised and the body refrigerated as soon as possible after death. In general, tissues should be recovered within the shortest possible period from the time of death. Standards vary around the world, depending on the tissue and the processing method to which it will be subjected.

Minimising bacterial contamination is further ensured by staff wearing sterile clothing and applying an aseptic technique during the tissue recovery process. This includes cleaning the donor using surgical detergents, alcohol wipes and sterile water; shaving the incision and skin retrieval areas; and draping the donor body before commencing the retrieval. Single-use equipment is employed where possible.

Tissue Processing

Tissue grafts are processed to improve safety and efficacy and for long-term storage of the donated material. There are multiple ways of processing, depending on the properties of the graft that need to be retained. The core methodology by which viable tissues (skin, heart valve, cardiovascular and meniscus grafts) are processed comprises dissection, decontamination by antibiotic cocktail and cryopreservation. While it may be desirable to sterilise a graft to increase safety, this is not practical where retention of donor cell viability is required. For many types of tissue allograft, in particular musculoskeletal allografts, the presence of viable cells is not required, and in these cases processing reduces the risk of disease transmission by removal of blood and marrow and by reducing or eliminating contamination by chemical or physical means.

Each TE should have a policy for acceptance or rejection of tissues if certain organisms are detected in bacterial screening during different stages of processing. The policy should be based on the pathogenicity of the organism and the validated effectiveness of any subsequent decontamination or sterilisation steps. Most tissue processing involves the use of a clean room with tissue operators working within standard working hours; however, several European and international tissue banks are investigating the use of automated closed systems that would allow tissue processing over a 24-hour period with minimal staff intervention. Not only could this reduce processing times, it could also reduce the potential for contamination.

Femoral heads from living donors removed during surgery in an operating theatre can be frozen and transplanted without further processing in the absence of bacterial or fungal contamination in validated tests.

Supply and Traceability of Tissues

Directive 2006/86/EC lays down the obligation for TEs to affix a 'Single European Code' (SEC) on tissues distributed for clinical application in the EU, which facilitates tracking of tissue from the donor to the recipient. Most TEs supply tissues direct to operating theatre departments, and it is the responsibility of the receiving hospital to track from the receipt of the tissue to the graft's ultimate fate. Many TEs supply the hospital with a recipient record to be completed for each graft and returned to the TE. The users should always be advised to do the following:

- Keep a log of tissue received and used.
- Record any allograft unit numbers in the patient's notes.
- Inform the tissue bank immediately of any adverse reaction that might be attributable to the tissue graft.

In many cases, tissues are supplied for specific cases and stocks are not held locally, but some units prefer to keep stocks of tissue immediately at hand for use in emergency or unexpected cases. Depending on the type of tissue, the hospital may require a licence in the EU to store tissue grafts for more than 48 hours; tissues containing donor cells such as cryopreserved skin grafts require a licence for storage, whereas acellular tissues, such as processed freeze-dried bone grafts, do not.

Clinical Applications

Tissue allografts are used in a variety of clinical indications in orthopaedic, spinal, cardiac, vascular, ophthalmic and plastic and reconstructive surgical procedures. Some of them are listed in Table 46.1. The clinical demand for tissues varies depending on surgical practices in different countries. Additionally, certain types of graft, for example heart valves and meniscal cartilage, need to be closely size-matched to the recipient. Taken together with donor age limits applied to certain types of graft, this creates a situation where some types and sizes of graft may be in short supply. National, European and international collaboration is one potential solution to this issue.

Serious Adverse Events and Reactions

Directive 2006/86/EC requires Member States to have systems for reporting adverse reactions and events related to the procurement, testing, processing, storage or distribution of the tissue, which might lead to the transmission of communicable disease, death or life-threatening, disabling or incapacitating conditions that might result in or prolong hospitalisation or morbidity in the recipient. Many of these case reports are included in the NOTIFY library [3]. In the UK, the HTA has developed an electronic reporting system for tissue and cell facilities, in line with the requirements of the Directive. In addition to incidents that may directly affect patients due to the compromised safety or quality of the tissue, TEs must also report incidents that may impact donors or their families.

Advances in Tissue Processing and Regenerative Medicine

Almost all traditional tissue processing involves retention of donor cells, either living or dead, but recent developments indicates that only a few tissue allografts require living donor cells, e.g. cryopreserved skin, corneas, living articular cartilage allografts. In all other circumstances,

Table 46.1 Indications for tissue allografts.

Types of graft	Surgical specialty	Surgical procedure (examples)
Heart valves	Cardiac	Heart valve replacement
Tendons and ligaments	Knee surgery	Ligament reconstruction
Meniscus	Knee surgery	Replacement of damaged meniscus (in selected cases)
Frozen femoral head, morcellised bone grafts	Orthopaedic (hip and knee)	Impaction grafting at revision joint surgery
Massive bone allograft	Orthopaedics	Post trauma or tumour excision reconstruction
Demineralised bone	Spinal surgery, orthopaedic, oral and maxillofacial	Spinal fusion, non-union or trauma defects, to fill cysts and tumour cavity defects
Cornea	Ophthalmology	Keratoconus, corneal ulcers, trauma, chemical burns
Skin	Burns	Burns, toxic epidermal necrolysis
Decellularised dermis	Plastic and reconstructive, breast surgery, abdominal surgery	Chronic wounds, breast reconstruction, abdominal wall repair
Blood vessels	Vascular	To replace infected prosthetic graft, lower-limb ischaemia

presence of donor cells may be detrimental to allograft incorporation or may delay incorporation of the graft. Regenerative medicine uses techniques of tissue engineering to remove donor cells without affecting the biological, biomechanical or biochemical parameters of the tissue [4].

Decellularised Tissues

Decellularised tissue has been available as an allograft since 1995 and several tissue banks now offer decellularised dermis and decellularised heart valves to surgeons. A major advantage of decellularised tissue becoming repopulated by recipient cells is that, over time, the grafted tissue becomes remodelled by the recipient cells, the donor extracellular matrix is replaced with recipient matrix and the allograft becomes part of the host. Two consequences of this are:

- A reduction in immune and/or inflammatory response.
- The ability of the grafted/remodelled tissue to grow and be able to repair itself as part of the recipient.

In addition to improving tissue incorporation, grafting of decellularised heart valves leads to a reduction in complications and the need for further operations with time when compared to conventionally cryopreserved valves [5].

Limbal Stem Cells

The ability to add cells to banked tissue allografts is a major step forward in regenerative medicine treatment. Amniotic membrane has been used as a conventional allograft to treat severe ocular surface diseases for several years, owing to its ability to facilitate corneal re-epithelialisation and reduce scarring and inflammation; however, more recently amniotic membrane has been used as a substrate on which epithelial stem cells can be expanded prior to transplant. The epithelial stem cells are derived from biopsies of the limbal region of the corneum; the stem cells can locate to stem cell niches when transplanted and thus provide a long-term solution to limbal stem cell deficiency. Limbal stem cells can be obtained either from the patient or from a donor. Several independent tissue banks and clinical research units have worked on methods for *ex vivo* expansion of limbal stem cells on matrices for many years. Recently a method using a fibrin scaffold has been licensed by the European Medicines Agency (EMA) for selected clinical indications [6]; this is an expensive treatment and has prevented or delayed independent research and development.

Other Developments

Regenerative medicine opens up the possibility of replacing almost every damaged or worn-out tissue with a new tissue capable of becoming part of the patient and returning normal functionality. Further advances include using decellularised tissue as a starting material for production of human tissue hydrogels (tissues that are fluid at 4 °C but gel at physiological temperatures [7]) or bio-inks for 3D printing. These hydrogels, or the powders used to produce them, can have their biomechanical and biochemical properties modulated by incorporation of bioactive factors and/or biocompatible polymers [7,8].

Conclusion

The banking of tissues is increasing within blood services, where expertise in donor selection, donor testing and quality management is being applied to the banking of tissues. For deceased donors, a thorough medical and behavioural history from a number of alternative sources is recorded to compensate for the lack of a direct donor interview. Tissues should be retrieved within the shortest possible time period after death to minimise the risk of bacterial contamination. Minimising bacterial cross-contamination is ensured by applying aseptic retrieval techniques. Tissue processing is necessarily open and usually involves decontamination or terminal sterilisation. Traceability is an essential aspect of the quality chain and should ideally be supported by machine-readable identification codes. Tissue banking and processing are part of a rapidly evolving field and are becoming more and more related to personalised regenerative medicine.

KEY POINTS

1) Tissue grafts are used in surgical procedures to replace damaged or lost tissues in patients. Most tissue allografts are donated by deceased donors and some by living donors undergoing surgery.
2) Selection criteria for donors are based on an analysis of risks and benefits related to application of specific tissues.
3) Processing tissue reduces the risk of disease transmission by removing blood and marrow and by reducing or eliminating contamination by chemical and physical means.
4) Tissue allografts have been used in surgical procedures for many years with great success, but with known limitations. Regenerative medicine using decellularised and tissue-engineered allografts may allow full incorporation of the graft into the patient, such that it becomes part of the patient and is able to grow and repair itself.

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Further Reading

Cell and Tissue Banking, a specialised journal covering all aspects of the field. https://www.springer.com/journal/10561

European Directorate for the Quality of Medicines and Healthcare. Guide to the Quality and Safety of Tissues and Cells for Human Application, 4th edn. https://www.edqm.eu/en/organs-tissues-and-cells-technical-guides

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47 Observational and Interventional Trials in Transfusion Medicine

Alan T. Tinmouth[1,2], Dean Fergusson[2] and Paul C. Hébert[3]

[1] *Division of Hematology, Department of Medicine, Ottawa Hospital, Ottawa, Ontario, Canada*
[2] *Ottawa Hospital Center for Transfusion Research, Clinical Epidemiology Program, Ottawa Health Research Institute, Ottawa, Ontario, Canada*
[3] *Department of Medicine, Centre Hospitalier de L'Université de Montréal, Centre Recherche du Centre Hospitalier de Montréal, Montréal, Québec, Canada*

Randomised controlled clinical trials (RCTs) are the 'gold standard' clinical research design used to distinguish the risks and benefits of therapeutic interventions. In 1948, for the first time a controlled clinical trial made use of random allocation, a control group and blinding. Additional principles guiding the design of RCTs were first elaborated by Sir Austin Bradford-Hill in the 1960s [1].

Many important questions regarding the use of blood products and alternatives such as blood conservation therapies have not been the subject of well-designed and executed RCTs. Clinicians frequently base decisions on suboptimal levels of clinical evidence. Their reasons for the relative paucity of large clinical trials in transfusion medicine include:

- Transfusion medicine has historically been a laboratory-based specialty with research focused on the product.
- Blood components are a supportive treatment for patients under the care of other physicians whose research focus is directed at the treatment of the underlying disease.
- The impact of supportive therapy with blood components on important clinical outcomes may be difficult to measure.
- Obtaining funding for research of a supportive, as opposed to a curative, therapy is often difficult.
- Blood components have been part of standard care for years without good evidence to define the benefits or harms, or specific indications of use.
- Few industry partners are willing to invest in large clinical trials given that products are already in wide use.

In this chapter, we outline some of the methodological issues central to the development and conduct of observational and interventional trials, including RCTs, in transfusion medicine.

Types of Clinical Studies

To ascertain the effectiveness of an intervention, the RCT remains the preferred study design, as it should minimise the most important biases if properly conceived and executed.

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Despite being the 'gold standard', there are often practical, legal, financial and ethical limitations to the use of clinical trials. While many of these limitations have been well described, one unique obstacle in transfusion medicine is the conduct of an RCT when an intervention is universally implemented, such as a new processing method or testing procedure for the entire blood supply. By implementing an intervention such as universal prestorage leucocyte reduction, an RCT becomes impossible within that population. If an RCT is not possible, other study designs need to be considered, including quasi-experimental and observational designs. The strengths and weaknesses of individual study designs are critical in selecting the most appropriate study design to answer a specific research question (Table 47.1).

Observational Studies

Two types of observational designs are often considered in clinical research: case–control studies and cohort or prognostic studies (Figure 47.1). In all observational studies, the first step is to define (1) the research hypothesis, (2) the population, (3) the exposure(s), (4) the outcome(s) and (5) the covariates (factors other than the exposure that may influence the occurrence of the outcomes).

Table 47.1 Strengths and weaknesses of different study designs.

Study type	Strengths	Weaknesses
Observational		
Case–control study	• Can assess rare outcomes with longer latency period • Can assess multiple risk factors • Can be completed quickly • Least costly	• Prone to recall, selection and other biases • Inefficient when assessing rare risk factors • Cannot determine incidence or prevalence • Cannot determine causation
Retrospective cohort study	• Can assess multiple exposures and outcomes • Can calculate incidence and prevalence • Helpful groundwork for future RCT • Less costly, especially if using administrative data	• Unable to account for unknown confounders • Inefficient when assessing rare outcomes or long latency period • Data may be poor, missing or absent • Cannot determine causation
Prospective cohort study	• Can assess multiple exposures and outcomes • Can calculate incidence and prevalence • Reduces selection bias	• Unable to account for unknown confounders • Inefficient when assessing rare outcomes or long latency period • Potential loss to follow-up • Cannot determine causation • Can be costly and time-consuming
Quasi-experimental		
Before/after cohort study	• Can assess implementation of policy/procedure change • Simple design • Control groups can account for secular and sudden changes	• Uncontrolled studies prone to secular and sudden changes unrelated to intervention • Hawthorne effect (non-specific beneficial effect of taking part in research)

Table 47.1 (Continued)

Study type	Strengths	Weaknesses
Interrupted time series	• Can assess implementation of policy/procedure change • Analysis accounts for temporal changes	• Requires data collection at multiple time points before and after intervention • Still prone to effect of other events at time of intervention
Randomised Controlled Trials		
Parallel group trial	• Simplest RCT for design and analysis • Useful to establish either efficacy or effectiveness	• Most widely used and trusted • Establishing efficacy and effectiveness generally requires more than one RCT
Factorial design	• Assesses multiple treatments in a single experiment • Properly powered, can assess for the presence of interactions	• Inefficient if weak to moderate interaction between interventions
Crossover trial	• Increased statistical efficiency as each patient acts as their own control	• Susceptible to carryover effects • Conditions must be stable • Requires adequate washout period between treatments • Treatment must have rapid effect • Outcomes must rapidly respond to treatment changes • Cannot assess meaningful long-term outcomes
Cluster trial	• Assesses new policy or initiative, particularly complex intervention • Avoids contamination between individuals within cluster • Useful when individual randomisation is impossible or impractical	• Less efficient compared to individual randomisation (increased sample size) • Requires adequate number of available clusters
Step-wedge cluster trial	• Allows for sequential rollout of intervention • Ideal for complex programmes and interventions, allowing minimum interference with implementation • Useful if carryover effects • Repeated measures reduce sample size • All groups/patients offered the intervention	• Prolongs duration of trial • Susceptible to temporal changes during rollout • Complex analysis • Can require rapid recruitment and outcome collection

RCT, randomised controlled trial.

In a case–control study, a group of individuals with an outcome (cases) and another group of non-affected individuals (controls) who would be considered at risk of developing the outcome are identified. The investigators then seek to identify potential risk factors in both groups. This classic epidemiological design is a retrospective study that is ideally suited to the investigation of rare diseases and the identification of potential aetiological or risk factors, particularly if there is a long latency period [2]. In transfusion medicine, case–control studies would be ideally suited for the initial study of

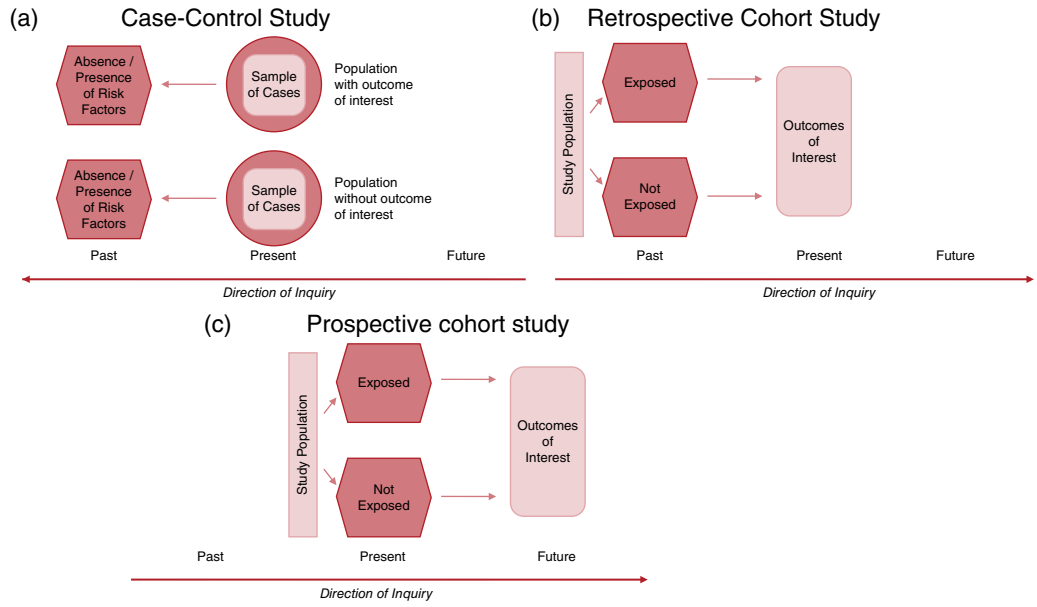

Figure 47.1 Observational study designs: Case-control studies and cohort study designs including the timing and direction of inquiry. *Source*: Tay J, Tinmouth A. Observational studies: what is a cohort study? Transfusion 2007;**47**(7):1115–17. Reproduced with permission of John Wiley & Sons.

rare conditions such as transfusion-related acute lung injury (TRALI) or the association between blood transfusion and variant Creutzfeldt–Jakob disease (vCJD). For example, Silliman et al. were able to identify using a case–control design that certain diagnoses (haematological malignancies and cardiac disease) and the age of the platelets were associated with TRALI [3]. Case–control studies can be an efficient, cost-effective study design; however, they are difficult to do well and fraught with potential biases including selection bias for cases and controls, and recall and observer bias related to collection of information. A case–control design will provide odds ratios as an estimate of the association between an exposure and an outcome, but cannot determine the incidence of a disease and does not allow for causation to be determined.

A second observational design choice is a cohort study. Whether retrospective (data already collected) or prospective (data to be collected), cohort studies follow patients forward in time and evaluate outcomes based on a known exposure, risk factor or treatment. In this type of study, individuals are identified well in advance of developing either a disease or the outcome of interest, and followed forward in time. A cohort study may provide important clues to the aetiology of a disease or health state by comparing individuals who develop the disease and those who do not. It may also lead to a better understanding of the incidence of the disease or, in patients with an established disease, the course of the disease, the prognostic information or the effectiveness of treatments. This design is most powerful when all eligible individuals are identified early and followed prospectively throughout the course of the study without any losses to follow-up.

The positive and negative attributes of cohort studies can be illustrated by some of the cohort studies that have examined the relationship between anaemia, red-cell transfusion and mortality. A retrospective study of Jehovah's Witness patients conducted by Carson and colleagues demonstrated a clear association between increasing degrees of preoperative anaemia and mortality rates in the presence of ischaemic heart disease [4].

Given that blood transfusions were not part of the care, the clinical consequences of anaemia were not affected by transfusion practices, allowing observations without biases from treatment. This is likely the best possible study and data, as a prospective RCT examining the question would not have been ethical or feasible.

In contrast, multiple cohort studies reported an association between prolonged red-cell storage and adverse outcomes. These observational studies all had major limitations, particularly the interdependence of anaemia, co-morbidities and transfusions. This is a classic example of a common bias affecting observational studies known as confounding by indication. This is the mixing or blurring of effects where an outcome has an association with an exposure, but the effect is caused by a third factor that is related to both the exposure and the outcome [5,6]. Subsequent large RCTs did not find an effect of prolonged red-cell storage on mortality or other adverse outcomes, highlighting the potential biases and limitations of observational cohort studies, and the inability of this study design to demonstrate causation or draw definitive conclusions.

The use of quasi-experimental studies can be of particular value in the evaluation of a universally implemented intervention such as prestorage leucocyte reduction. In such a case, either subjects must be sampled over a period of time prior to and after the implementation of the programme (a 'before-and-after' or interrupted time-series study), or sampling must occur among subjects who received leucocyte-reduced blood products and another population that did not receive such products (a standardised incidence study). In a before-and-after study design, the frequency of an outcome in a specified population is measured first during a period of time when the exposure is absent, then in the same population during a period of time where exposure is present. Consecutive periods before and after the implementation of a treatment are often compared. When a single measurement in both the pre- and postintervention periods is compared, there is the risk that changes occurring as a result of other ongoing factors may be attributed to the intervention. To limit this temporal bias, the changes in the experimental group may be compared to a control group not exposed to the intervention (controlled before-and-after study) or determinations of the outcome at multiple time points before and after the implementation of an intervention (interrupted time series) should ideally be used to account for secular or temporal changes.

Well-executed case–control studies may provide clues about the aetiology or risk factors associated with the development of a disease or complication. A cohort study may provide the best estimate of incidence, prognosis and risks associated with the development of a disease or its complications. Standardised criteria to assess the quality of observational studies are available [7]. Both observational designs provide weak inferences regarding specific therapeutic interventions, because many forms of bias and confounding remain even after complex multivariable analysis. Inherent in both case–control and cohort studies is the inability to determine causality between a risk factor or treatment and a specific outcome. Before-and-after studies and time-series analysis, both quasi-experimental designs, may provide some inferences regarding clinical consequences attributed to the implementation of a universal programme when an RCT is not possible [8].

Randomised Controlled Trials

Overall Design Approaches for Randomised Controlled Trials

For therapeutic interventions, the highest quality of evidence is provided by a well-performed RCT. However, there should be an awareness that RCTs may be complex in terms of both their design and their execution and, therefore, usually require significant

resources to complete. Observational studies, meta-analyses and surveys of practice are important foundations prior to starting an RCT. A pilot study to assess feasibility can also be invaluable to ensure the ultimate success of a large RCT. In this section, a conceptual framework is provided for RCTs that should assist providers and consumers of clinical research. The CONSORT statements provide checklists for assessment of the quality of RCTs [9].

The ideal RCT establishes whether a therapeutic intervention works, and determines the overall benefits and risks in a predefined patient population. Most commonly, an RCT evaluates whether an intervention is beneficial (superiority trial) compared to another treatment or no treatment. However, RCTs can also determine if an intervention is similar (non-inferiority or equivalency trial) to another treatment [10]. The influence of chance, bias and confounding on the difference in outcomes between the treatment groups is reduced by the design of the clinical trial, especially the random assignment of patients to the different treatments.

The ideal RCT should attempt to fulfil its objectives with the fewest patients possible (often termed 'statistical efficiency'). Unfortunately, these objectives tend to be in conflict and, more importantly, economic considerations frequently limit our ability to fulfil all these objectives. For instance, by maximising the efficiency of a study with a smaller study size, investigators might sacrifice their ability to draw conclusions in clinically important subgroups because of inadequate sample size.

The most important consequence of these conflicting objectives is that choices made in the design of RCTs must focus on whether an intervention works or whether it results in more good than harm for patients [11]. Trials that attempt to determine therapeutic *efficacy* address the question 'Will the therapy work under ideal conditions?' Trials attempting to determine therapeutic *effectiveness* address the question 'Will the therapy do more good than harm under usual practice conditions in all patients who are offered the intervention?' Clearly, both questions will yield useful information. Efficacy is often established first (e.g. phase III pharmaceutical studies), then the intervention may be evaluated for its effectiveness, though this occurs more rarely.

As the characteristics of efficacy and effectiveness trials can differ considerably, the planning of an RCT should reflect the design that will best reflect the primary study question (Tables 47.2 and 47.3). Efficacy trials attempt to maximise internal validity, defined as the extent to which the experimental findings represent the true effect in study participants. This is often at the expense of external validity, defined as the extent to which the experimental findings in the study represent the true effect in the target population. For effectiveness studies, external validity is usually emphasised over internal validity.

Table 47.2 Considerations in determining which design approach to implement in transfusion trials.

	Choice of design	
Criteria to consider	**Favouring efficacy**	**Favouring effectiveness**
Evidence	Limited evidence	Efficacy well documented
Importance of the question	Rare and less serious	Common and serious problem
Feasibility	Not demonstrated	Adequate accrual and confirmed feasibility
Risks	Unknown or significant consequences	Minimal or acceptable risks given benefits
Benefits	Limited or unknown benefits	Significant benefits anticipated

Table 47.3 Comparison of study characteristics using either an efficacy or an effectiveness approach when designing a study.

	Efficacy trial	Effectiveness trial
Study characteristics		
Research question	Will the intervention work under ideal conditions?	Will the intervention result in more good than harm under usual practice conditions?
Setting	Restricted to specialised centres	Open to all institutions
Patient selection	Selected, well-defined patients	A wider range of patients identified using broad eligibility criteria
Study design	Smaller RCT using stringent rules	Large, multicentre RCT using simple rules
Baseline assessment	Elaborate and detailed	Simple and clinician friendly
Intervention	Tightly controlled optimal therapy under optimal study conditions	Less-controlled therapy administered by investigators using accepted approaches
Treatment protocols	Rigorous and detailed	Very general, follows standard practice outside of the study intervention
Compliance	Non-compliance not tolerated	Non-compliance tolerated
Endpoints	Disease related Related to biological effect Surrogate endpoints	Patient related, such as all-cause mortality or quality of life
Analysis	By treatment received (per protocol) Non-compliers removed	Intention to treat All patients included
Data management		
Data collection	Elaborate	Minimal and simple*
Data monitoring†	Detailed and rigorous	Minimal

* Data capture can be electronic using pre-existing databases.
† Data monitoring refers to the review of source documents and adjudication/verification of outcomes.
RCT, randomised controlled trial.

An example of an efficacy trial is the Stroke Prevention in Sickle Cell Anemia (STOP) trial, which compared the exchange transfusions to prevent stroke in 130 paediatric sickle cell patients with abnormal transcranial Doppler studies [12]. In contrast, the Clinical Randomization of an Antifibrinolytic in Significant Haemorrhage (CRASH-2) study was a large effectiveness trial that showed small but clinically important differences in all-cause mortality in 20 000 trauma patients receiving tranexamic acid [13]. Recent examples of large pragmatic effectiveness trials have sought alternatives to traditional written informed consent such as verbal consent or waivers, and have collected data from existing electronic databases, which decreases costs and allows for increased efficiency [14,15].

Many trials opt for a hybrid approach between large simple trials and tightly controlled clinical studies. The Transfusion Requirements in Critical Care (TRICC) trial [16], which allocated 838 critically ill patients to either a restrictive or liberal transfusion strategy, and the Platelet Dose (PLADO) trial [17], which randomised 1272 patients with hypoproliferative thrombocytopenia to low-, medium- or high-dose prophylactic platelet transfusions, provide examples. Zwarenstein and colleagues have proposed a series of criteria in the PRECIS 2 extension of CONSORT, arguing that most clinical trial characteristics are on

a continuum between being truly pragmatic (without any limitations) to being tightly controlled in explanatory trials [18].

Randomised Controlled Trial Design Alternatives

Once investigators have chosen whether an efficacy, effectiveness or hybrid approach will best answer the research question, there are several design options that may be considered (see Table 47.2).

Parallel Group Design

A two-group parallel design is the most common RCT design choice (Figure 47.2a). In this design, patients are randomly allocated to one of two therapeutic interventions and followed forward in time. It is the simplest to plan to implement, analyse and, most importantly, interpret. Therefore, a parallel group design is the most frequently adopted RCT design. Parallel group designs may also be used to independently compare three or more treatments [19].

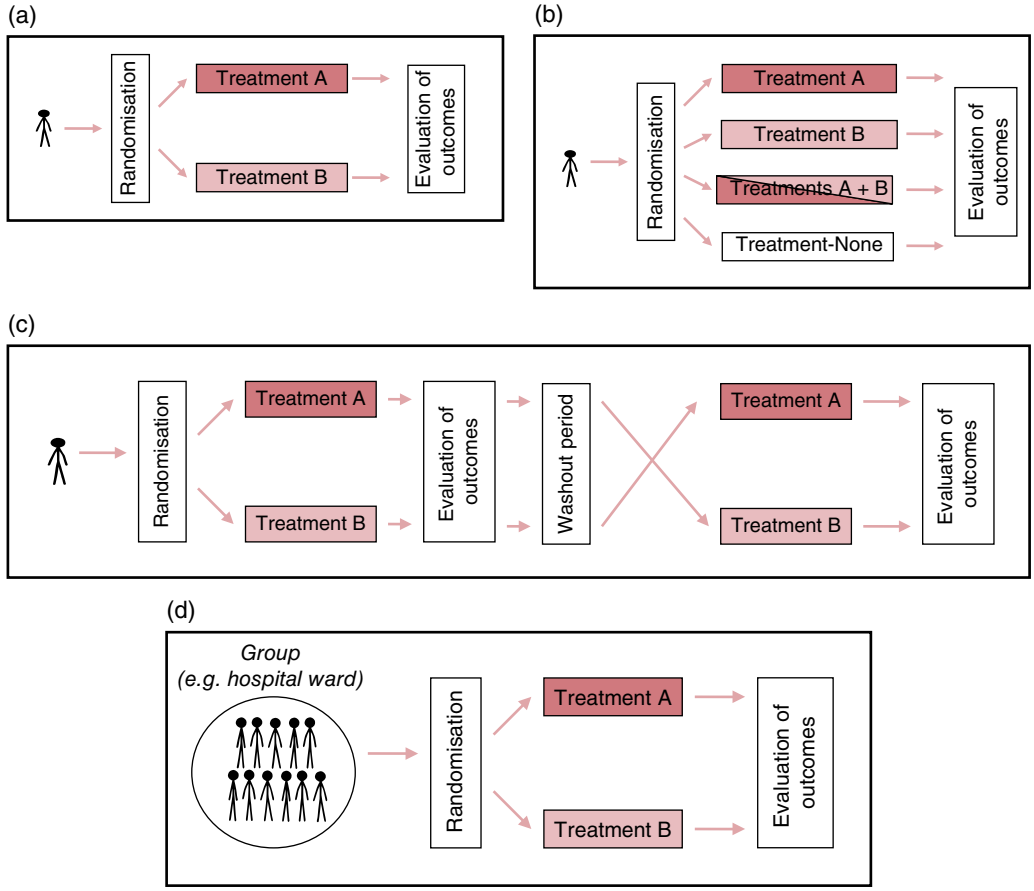

Figure 47.2 Randomized controlled trial design alternatives. (a) Randomised two-group parallel design: subjects randomly assigned to treatment A or B. (b) Factorial design: all subjects randomly assigned to treatment A, treatment B, treatment A + B or no treatment. (c) Randomised crossover design: subjects randomly assigned to treatment A followed by treatment B (after washout period) or treatment B followed by treatment A. (d) Randomised cluster design: all subjects in one group/area (e.g. by physician, by hospital, by ward) are assigned to treatment A or B. *Source*: Adapted from Tinmouth A, Hébert P. Interventional trials: an overview of design alternatives. Transfusion 2007;**47**(4):565–7.

Factorial Design

The use of factorial designs may also be considered when a number of therapies are being evaluated in combination. For instance, in a 2 × 2 factorial design, two interventions are tested both alone and in combination, and compared with a control group (usually a placebo; Figure 47.2b) [20]. This means that investigators can efficiently test two interventions with only marginal increases in sample size. In addition, the benefits of treatment combinations can be evaluated in a controlled manner. This design is most useful when interactions are either very strong or non-existent [20]. Traditionally, the factorial design is used to answer two separate study questions. For example, Robertson and colleagues randomised traumatic brain injury patients to (1) erythropoietin or placebo and (2) a restrictive or liberal transfusion threshold, evaluating the effectiveness of therapies on mortality [21].

Crossover Design

Another RCT design option particularly amenable to an efficacy evaluation is a two-period crossover study, in which patients are used as their own controls. In this trial, patients are randomised to one of two therapies for a fixed period of time and then proceed to receive the other therapy in a second comparable interval (Figure 47.2c). Minimising 'between-subject' variability in this manner makes significant gains in efficiency and reduces the sample size. Crossover studies are therefore best suited to relatively stable conditions (stability is required during the study), interventions with rapid onset of action and a very short half-life to ensure an appropriate washout period (the biological effect must disappear prior to the second treatment period), and rapidly modifiable endpoints [20]. MacLennan et al. used a crossover design to compare the efficacy of platelets stored for 6–7 days versus 2–5 days on the proportion of days with clinically significant bleeding [22].

Cluster Design

All designs discussed so far have described the evaluation of interventions for individual patients. However, it is sometimes necessary to evaluate therapies, protocols, guidelines or treatment programmes for groups of individuals. Using this design, groups or 'clusters' such as intensive care units (ICUs), wards, hospitals and physician practices are randomised to receive an intervention or control (Figure 47.2d). Cluster design may be the most appropriate for evaluating complex or multidimensional interventions such as the implementation of care paths, educational interventions, transfusion audits or other interventions to change transfusion practice. For these evaluations, the cluster is a more natural method of allocation than the individual [8]. Cluster trials are advantageous, as there could be a risk of contamination such that the intervention will be implemented in all patients rather than only the patients assigned to receive the therapy, which would bias the results of the study. However, the allocation of interventions to groups rather than individuals will increase the sample size as a result of the non-independence within the group, and it is often difficult to infer what happened at an individual level. An additional concern in cluster trials is the possibility of large variations between clusters that may make it difficult to detect actual differences between therapies [23].

As an example of a cluster randomised trial, Murphy et al. randomised wards at different hospitals to receive units of red cells with labels reminding nurses to check the patient and component identification [24]. The randomisation by wards was important to ensure that nurses transfusing red cells without the reminder were not those who had been previously exposed to the reminder tags. In a variation of a cluster trial, Karkouti et al. used a stepped-wedge cluster design to evaluate the use of point-of-care haemostatic testing and a transfusion algorithm in cardiac surgery [25]. This study design is particularly well suited to complex programmes or interventions. The intervention is randomly and

sequentially introduced in all hospitals, and the results are compared prior to and following the introduction of the protocol at each hospital and between hospitals.

Selecting a Study Population

The choice of study population will invariably depend on the study question, the underlying hypothesis and a number of other factors. The choice of a hypothesis that will address either therapeutic efficacy or effectiveness will have a substantial impact on the selection of the study population and the overall design of the study (Table 47.3) [11]. In choosing an efficacy approach, investigators usually perform the study in a well-defined patient population (using restrictive eligibility criteria and disease definitions) where the intervention has the highest probability of demonstrating an effect. This will decrease the overall variability attributed to patient selection, but may have adverse consequences on patient recruitment and jeopardise the generalisability of study results. When defining the eligibility criteria for an effectiveness trial, investigators should utilise more liberal criteria in a wide range of clinical settings. The study population may also be affected by other factors such as the spectrum of biological activity or the participation of specialised centres for recruitment.

Selecting the Comparator

In an RCT, an intervention is compared to either another treatment or no treatment. If there is a known effective treatment or standard of care, this should be the alternative treatment or comparator. When there is no known effective therapy, then the comparator should be no treatment, which is commonly defined as 'usual care'. Ideally, the intervention(s) should be blinded so that none of the investigators, patients, clinical or research staff is aware of an individual patient's treatment assignment. In the case of no treatment, a placebo can be given to maintain blinding. This may be possible with blood products when repeated dosing or monitoring of response is not required. One of the RCTs evaluating convalescent plasma for COVID-19 used a placebo control [26]. Unfortunately, in RCTs in transfusion medicine, it is often not feasible to blind the intervention(s), particularly if a blood transfusion is being compared to no treatment. In such cases, the research staff assessing and collecting the outcome should be blinded to the treatment allocation.

Selecting Outcomes

In most clinical trials, the clinical investigative team should consider a number of potential outcomes, both fatal and non-fatal (Box 47.1). An outcome is defined as a measurement (e.g. haematocrit) or an event (e.g. death) potentially modified following the implementation of an intervention. If all are given equal consideration, concerns arise about multiple comparisons and interpretation of a study with heterogeneous findings. Thus, it is important to choose a primary outcome that will determine an intervention's therapeutic success or failure. Secondary outcomes will provide supportive evidence in secondary analyses and assess potential adverse outcomes. The primary

Box 47.1 Guides to the choice of outcome measure in a randomized controlled trial.

- Is the outcome causally related to the consequences of the disease?
- Is the outcome clinically relevant to the healthcare providers and/or patients?
- Has the validity of the outcome (for complex outcomes such as scoring systems or composite outcomes) been established?
- Is the outcome easily and accurately determined?
- Is the outcome responsive to changes in a patient's condition?
- Is the outcome measure potentially able to discriminate between patients who benefit from a therapy and patients in the control group?

outcome is also essential in determining the sample-size requirements in a clinical trial.

There are a number of factors that should be considered prior to the selection of outcomes for a study. The primary outcomes should be clinically important and easily ascertained. By fulfilling these two criteria, the investigator will have a much greater chance of influencing clinical practice once a study has been completed and published. Outcomes should also measure what they are supposed to measure (validity) and be precise and reproducible. An outcome must be able to detect a clinically important true positive or negative change in the patient's condition following a therapy.

The sample size in a clinical trial comparing two therapies is based on the baseline event rate, the expected incremental benefit or difference (superiority trial) or the maximum accepted difference that would still be considered equivalent (inferiority trial), the level of significance (α) and the power to detect differences ($1 - \beta$). Establishing the incremental benefit of a new therapy or the margin of non-inferiority is vitally important because of the enormous sample-size repercussions. A sample-size calculation for an RCT requires that the investigators establish the minimum therapeutic effect detectable within the trial. This difference in outcomes between interventions is referred to as the minimally important difference (MID) or minimal clinically important difference (MCID) [8]. The MID is essentially establishing the level of discrimination in the study population who are exposed to the interventions given acceptable levels of error of type I (finding a difference when one does not truly exist) and type II (not finding a difference when one truly exists) and the baseline event rate. Too often, investigators calculate a sample size based on very large and unrealistic expected differences in outcomes.

To determine a plausible effect size, investigators should ask themselves the following questions:

- What difference or incremental benefit can be realistically expected of the experimental therapy? (Anticipated biological effect of therapy.)
- What is the smallest clinical difference in outcomes that would be clinically important? (Determine the minimal important difference or margin of non-inferiority.)
- Are the required number of patients available to participate in the clinical trial? (Feasibility.)
- How much of a benefit, given the added costs and expected adverse effects of therapy, would be required for clinicians, patients and administrators to adopt a new therapy? (Overall benefit of therapy.)

Investigators need to consider whether the absolute incremental benefit predicted is likely attainable using the experimental therapy. If not, another, more discriminating outcome should be sought.

Frequently, the treatment effect or difference in the desired clinical outcome is small. As a result, a surrogate or composite outcome may be chosen as the primary outcome for a trial to reduce the sample size. A surrogate outcome is defined as a laboratory or physical measure that accurately reflects a clinically meaningful outcome, and therefore can act as a substitute outcome with the goal of reducing the sample size [27]. A composite outcome combines more than one individual outcome. The latter may increase statistical efficiency and can combine multiple endpoints that are equally important [28]. Both surrogate and composite outcomes must be used judiciously and the results interpreted with caution [27,28]. Surrogate endpoints should clearly predict the clinical outcome, which often may not be the case (e.g. corrected count increment and bleeding in platelet transfusion trials) [27,29]. Composite outcomes must be related and, equally important, biologically plausible, uniform in their anticipated direction of effect and clinically relevant [28,29].

Analysis

The analysis of all RCTs testing the superiority of one intervention versus another should

initially assess the outcomes based on the planned intervention, regardless of whether the patient received the treatment (intention to treat). Removing patients from the analysis or changing the assigned group will potentially undermine the benefits of randomisation to help balance unknown confounders in the treatment groups. Undertaking a per-protocol analysis will also be important to fully answer the question of efficacy. In effectiveness trials, a per-protocol analysis is less important, but may still provide some insights into efficacy. In trials evaluating the non-inferiority of one intervention versus another, a per-protocol analysis should be the primary analysis, as losses to follow-up and non-adherence to interventions will introduce too much noise. The goal of this analysis is to ensure that any treatment effect is not missed, which ensures that conclusions are as robust as possible having looked to exclude any possible difference. Subgroup analyses can also be performed to evaluate any differential effect (interactions) of the intervention on specific patient groups. While these can be valuable in generating further hypotheses, they are often not sufficient to determine causality unless the randomisation is stratified for the subgroup factor.

Conclusion

Randomised controlled trials remain the 'gold standard' to evaluate therapeutic interventions, but many aspects of transfusion therapy have not been evaluated in well-designed and executed clinical trials. Given the expense and difficulty of performing RCTs, careful attention to the study design is required prior to enrolling any patients (Box 47.2). Different design alternatives, such as factorial designs or cluster randomisation, should be considered to ensure that the study design is optimal to address the research question. Decisions regarding the study population and outcomes must also consider the primary objective of the study question and feasibility.

Although RCTs provide the most unbiased and accurate assessment of the efficacy and effectiveness of therapeutic and preventive interventions, they remain challenging and expensive to conduct. As performing RCTs is not always feasible due to logistical and

Box 47.2 Suggestions for planning a randomized controlled trial (RCT) in transfusion medicine.

- Explicitly determine whether you are primarily interested in establishing therapeutic efficacy or effectiveness.
- Whenever possible, undertake an RCT as part of a broader research programme.
- If the study intervention is complex (or risky) or if other aspects of study feasibility are questionable, a pilot study should be considered.
- Whenever possible, investigators should use simple rather than complex designs (two-group parallel design vs factorial design).
- The study population should be tailored to the intervention.
- Ideally, the study intervention and treatment protocols should not aim to substantially modify or affect usual clinical practice.
- Given the complexity of RCTs, data collection should aim to clearly describe the study population, co-interventions and all major study outcomes.
- In choosing primary study endpoints, investigators should focus on patient-oriented outcomes rather than surrogate or biological markers.
- If you are planning a seminal RCT, you may only have one chance to get it right. When making compromises, always opt to answer questions that most clinicians consider most important.
- In establishing the minimally important difference, select a potentially achievable but clinically meaningful benefit.

ethical constraints, observational studies including cohort and case–control studies can be useful study designs to evaluate specific outcomes; however, these studies are prone to bias. As more research groups form to address unanswered therapeutic questions in transfusion medicine, investigators will invariably better understand the strengths and limitations of different RCT and observational study design characteristics.

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KEY POINTS

1) Properly conducted randomised controlled trials (RCTs) are the best means to evaluate the risk and benefits of therapeutic interventions.
2) Observational studies can be useful when RCTs are not feasible: case–control studies are particularly useful to evaluate rare outcomes and cohort studies can examine outcomes following known exposures, risk factors or therapies. However, all observational studies are prone to bias and cannot show causation.
3) The design of an RCT depends on whether the investigators wish to evaluate the *efficacy* or the *effectiveness* of an intervention.
4) A two-group parallel group design is the simplest RCT to design, execute and evaluate, but alternative designs can be useful in specific circumstances.
5) Selecting the appropriate study population and the outcomes is critical to ensure both the feasibility of completing the RCT and the generalisability and clinical relevance of the study results.

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48 Getting the Most Out of the Evidence for Transfusion Medicine and Lessons from the COVID-19 Pandemic

Simon J. Stanworth[1], Susan J. Brunskill[2], Carolyn Dorée[2] and Yulia Lin[3]

[1] *NHS Blood and Transplant; Department of Haematology, Oxford University Hospitals; Radcliffe Department of Medicine, University of Oxford, Oxford, UK*
[2] *Systematic Review Initiative, NHS Blood and Transplant, Oxford, UK*
[3] *Precision Diagnostics and Therapeutics Program, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada*

What Is Meant by Evidence-Based Medicine?

Evidence-based medicine (EBM) has been described by Sackett et al. as 'the integration of best research evidence with clinical expertise and patient values' [1]. Proponents of EBM have particularly highlighted the nature of the evidence that is used to make clinical decisions, i.e. where is it from, how believable is it, how relevant is it to my patient and can it be supported by other data? However, evidence is only one of the factors driving clinical decision making, and clinicians will also need to consider the available resources and opportunities, individual patients' values and needs (physical, psychological and social), local clinical expertise and cost. In some situations, clinical judgement will determine that the available evidence for a specific problem is not applicable.

EBM is not just about comprehensively obtaining and evaluating clinical research evidence; it is also a means by which effective strategies for self-learning can be applied, aimed at continuously improving clinical performance. The focus of this chapter will be to discuss core elements of EBM with particular reference to clinical research in transfusion medicine, and to provide a practical approach to searching for evidence and critical appraisal, with some considerations of different study designs. The chapter also includes a review of how the evidence base for transfusion medicine was collated in response to the COVID-19 pandemic.

Levels of Clinical Evidence

Optimal evidence is the best evidence available to answer a research question. A key overarching objective in health research studies is to explore evidence of causality for interventions. While causality is extremely difficult (or impossible) to prove absolutely, hierarchical levels of evidence provide increasing graded confidence that an association exists. Data derived from randomised controlled trials (RCTs) continue to be

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regarded as the strongest support for evidence of efficacy or effectiveness.

In 1948, the first modern RCT in medicine was published, comparing streptomycin and bed rest for patients with pulmonary tuberculosis [2]. The authors chose to perform a controlled trial because 'the natural course of pulmonary tuberculosis is in fact so variable and unpredictable that evidence of improvement or cure following the use of a new drug in a few cases cannot be accepted as proof of the effect of that drug'. In that trial, assignment of patients to streptomycin or bed rest was done by 'reference to a statistical series based on random sampling numbers drawn up for each sex at each centre'. There were fewer deaths in the patients assigned to streptomycin (4 out of 55 patients) compared to bed rest alone (14 out of 52 patients) [2]. If the process of randomisation is done correctly, differences in outcome(s) between groups should be attributable to the intervention and not to other confounding factors related to the patient's demography, study setting or quality of care. This is illustrated by the table of baseline characteristics in a trial publication, which should be balanced for all patient features; in turn, this provides more confidence that other, unknown characteristics would also be balanced between trial arms and therefore any differences in trial outcomes are caused by the intervention.

The most common (and simple) design for an RCT is a parallel design, in which participants are randomly allocated to one of two groups. However, the RCT design comes with inherent challenges:

- RCTs are costly, especially if these studies are conducted at multiple centres and/or internationally.
- Small RCTs, or those that are inadequately powered, tend to overestimate the effect of the intervention and may place too much emphasis on those outcomes with more striking results.
- Small RCTs may be designed to detect unreasonably large treatment effects (which they will never be able to show because of their small size).
- RCTs with non-significant results may never be fully reported or found only in abstract form – a phenomenon known as publication bias.
- Effects of interventions tested by RCTs may be overgeneralised and inappropriately applied to different patient populations.
- RCTs are not suited to investigating low-frequency rare adverse effects, prevalence rates or diagnostic criteria.

In addition, a parallel RCT, as the most common form of trial design, will only address the research question for the study. While this is clearly the desired aim of the trial, it can appear to be quite inefficient and create more challenges for further research. As an example, a recently published trial of platelet transfusion in neonates compared two strategies for use of platelets; the PlaNeT-2/MATISSE trial [3] reported that a liberal prophylactic platelet transfusion regimen resulted in higher mortality and severe bleeding compared to a restrictive regimen. This important result has several implications, but the result only applies to the population defined by the inclusion criteria and may not be representative of many sick infants with co-morbidities. A further, wider question left unanswered is: What is the optimal platelet count for transfusion in neonates? This study only compared two thresholds, 25 versus 50×10^9/L. Harm was found with the use of the higher threshold, but it could be that thresholds lower than 25×10^9/L would be more optimal for babies. However, this would require a further study, which 'arbitrarily' may then pick a lower-threshold platelet count than 25×10^9/L. It could take a lot of time and resources to define the 'sweet spot' for platelet transfusion in preterm neonates.

One approach might be to explore novel trial designs, and the COVID-19 pandemic has seen the success of a range of alternative designs, including platform trials or multi-stage, multiarm designs. For example, the Randomised, Embedded, Multi-factorial, Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) defines an innovative adaptive trial design that can evaluate a number of treatment options

simultaneously and efficiently (www.remapcap.org) and has done so, most recently as seen during the COVID-19 pandemic.

In contrast to RCTs, observational studies, such as cohort or case–control studies, whether prospective or retrospective, may demonstrate an association between intervention and outcome; however, it is often difficult to be sure that this association does not reflect the effects of unknown confounding factors. The influence of confounding factors and biased participant selection can dramatically distort the accuracy of the findings in observational studies. This does not mean that findings from well-designed observational studies should be disregarded; such study designs can be very effective in establishing or confirming effects of large size. However, interpretation is more difficult when the observed effects are small. Clinical questions addressing possible aetiology, exploring questions of harm or monitoring adverse effects may be more suited to observational studies, such as the link between transfusion and cancer recurrence [4].

In order to identify any limitations in a study and understand the possible impact of these on the study findings and their overall interpretation, it is important for readers, and investigators gearing up to design their own studies, to know how to critically appraise the methodological quality of the research. Critical appraisal and evaluation will be discussed next.

Appraisal of Primary Research Evidence for Its Validity and Usefulness

One important component of EBM is the critical appraisal of the evidence generated from a study. Understanding how to critically appraise a study is a useful skill for all healthcare professionals, as it will help you establish whether the findings from a trial relate to one's own practice. Published studies should report sufficient detail pertaining to the study design, population, condition, intervention and outcome to allow the reader to make an independent assessment of the study by examining its methodological quality. Inadequate methodology and poor reporting of both study methods and study findings do not provide the needed reassurance to readers that patient selection, study group assignment and outcome detection were not biased, each or all of which may result in inaccurate inferences drawn from the study data.

Guidelines and checklists have been designed to help with the reporting [5,6], many of which can be found on the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) Network website (www.equator-network.org). The EQUATOR Network provides a coordinated framework to improve the reliability and value of published health research literature by promoting transparent and accurate reporting with a repository of different relevant reporting guidelines. Critical appraisal guidelines are also useful for authors of primary research because they define the information that should be included in their published reports.

The earliest critical appraisal tools were developed for RCTs. Box 48.1 provides details of some of the selected components of the critical appraisal process for clinical trials:

- The methodology of the study (participants, interventions and comparators, outcomes).
- The sample size.
- The methods used for the randomisation process.
- Whether research staff were blinded to treatment allocation and outcome assessment.
- The reporting of the results (the numbers randomised and the numbers analysed/evaluated, the numbers not available for analysis with reasons and the role of chance, i.e. confidence intervals).
- External validity.

One important aspect of clinical trial appraisal concerns the understanding of chance variation and sample-size

Box 48.1 Key components of the critical appraisal process for clinical trials.

Did the trial address a clearly focused issue?
Was the assignment of patients to treatments randomised?
Were patients, health workers and study personnel blinded to treatment allocation?
Were all the participants who entered the study accounted for within the results?
Were all the participants followed up and data collected in the same way?
Aside from the experimental intervention, were the groups treated equally?
How are the results presented and how large was the treatment effect?
How precise was the estimate of the treatment effect?
Were all the important outcomes for this patient population considered?
Can the results be applied to practice/different populations?

Source: Adapted from the Critical Appraisal Skills Programme worksheets (https://casp-uk.net).

calculation. The distinction is between 'no evidence of effect' and 'evidence of no effect': the former may be derived from results that are either underpowered or non-significant, whereas the latter implies a sufficient sample size to show superiority, equivalency or non-inferiority. Information about sample-size calculations should therefore be provided in the published reports of clinical trials. An additional source of information for a researcher on aspects of different study designs can be found in the research methodology section of the journal *Transfusion*.

Comparable standards can be applied to the critique of observational studies. The framework Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) can be used to explore the quality of the reporting of an observational study [7]. Another source is the Critical Appraisal Skills Programme (CASP) checklists (https://casp-uk.net/casp-tools-checklists), which support the critical appraisal of the methodological quality of a wide selection of observational study methodologies.

Reviews: Narrative and Systematic

Reviews have long been used to provide summary statements of the evidence for clinical practice. In general, all reviews can be narrative or systematic. Often written by experts in the field, narrative reviews provide an overview of the relevant findings, as well as being educational and informative. However, the content and summary of the evidence base in a narrative review will ultimately be based on what the authors feel is important.

On the other hand, a systematic review methodology sets out to gather the totality of the evidence on a subject and summarise it in an objective way using prespecified questions, robust methods for study identification and selection and detailed quality assessment and analysis. The aim is to be objective, transparent and explicit and to limit biases at all stages of the systematic review process. The output is a synthesis of the results of primary studies – a synthesis that is accessible to clinicians, researchers and policymakers alike. An additional type of review is a scoping review [8], often used to examine emerging evidence, to identify available evidence and knowledge gaps or to scope a body of literature prior to a more precise systematic review; a national network exists with resources to help improve the sharing of information and methodology (https://jbi.global/scoping-review-network).

Systematic reviews also form the background for clinical trial design by establishing what is currently known (and unknown), which methods were used to achieve that knowledge and what gaps remain. Systematic

reviews are not substitutes for adequately powered clinical trials, but should be considered as complementary methods of clinical research.

There are generally accepted 'rules' about how to undertake a systematic review, which include the following:

- Developing a team to undertake the systematic review, including clinicians who know the clinical area of the review and methodologists skilled in all aspects of the systematic review process.
- Developing a focused review question, clearly defining the Participants, Intervention(s) and Comparator(s) and the Outcomes of relevance to the review (the PICO criteria).
- Comprehensively searching for all material relevant to this question (Box 48.2 provides some practical suggestions for developing a more comprehensive search strategy).
- Using predefined, explicit criteria to assess eligibility and methodological aspects of identified studies.
- Reporting and explaining why studies were excluded.
- Using predefined, explicit methods for combining data from identified studies including, where appropriate, meta-analysis of the study data.

Box 48.2 Searching for reports of trials and clinical evidence.

Writing the Search Question and Selecting Search Terms

Construct your question as simply as possible, ideally by combining any two of the four parts of the PICO formula (Patient/condition; Intervention; Comparison; Outcome) and then adding any relevant synonyms and/or alternative spellings. For example, the question 'Are red cell transfusions effective in the treatment of hip fracture?' is best searched for as patient/condition AND intervention, which for a quick search in PubMed could be constructed like this:

> ((hip OR hips OR femur* OR femoral* OR acetabul* OR intertrochanteric OR subtrochanteric OR trochanteric OR pertrochanteric OR peritrochanteric) AND fracture*) AND (blood OR erythrocyte* OR red cell* OR red blood cell* OR RBC OR RBCs OR trigger* OR level* OR threshold* OR rule* OR restrict*) AND (transfus* OR hypertransfus* OR retransfus*)

Additional Tips for Searching in PubMed

- Use Boolean operators AND/OR to combine groups of search terms, but use NOT with care
- Use truncation to reduce the number of search terms used, e.g. bleed* OR haemorrhag* OR hemorrhag*
- For a narrow, targeted search, try searching by title alone, e.g. tranexamic acid[TI]
- For quick diagnostic or therapy searches, try using PubMed Clinical Queries: https://ncbi.nlm.nih.gov/pubmed/clinical

Choosing the Study Design/Hierarchy of Evidence

Search for information from the highest-level evidence, working down this list if there is little or no relevant evidence at higher levels:

1) Evidence from at least one **systematic review**
2) Evidence from at least one **randomised controlled trial**
3) Evidence from a well-designed observational study (e.g. **cohort or case–control studies)**
4) Evidence from well-designed non-experimental studies (e.g. **case series and case reports)**
5) Expert opinion (e.g. overviews, editorials, narrative reviews)

Box 48.2 (Continued)

Sources for Searching for Different Study Designs

Systematic Reviews and Randomised Controlled Trials

- Transfusion Evidence Library (Evidentia): www.transfusionevidencelibrary.com
- PubMed Clinical Queries (NLM): https://ncbi.nlm.nih.gov/pubmed/clinical – Category: Therapy; Scope: Narrow
- TRIP database: www.tripdatabase.com
- Cochrane Library (Wiley): www.cochranelibrary.com

Ongoing Clinical Trials

- WHO International Clinical Trials Registry Platform: http://apps.who.int/trialsearch
- ClinicalTrials.gov: https://clinicaltrials.gov

Observational Studies

https://ncbi.nlm.nih.gov/pubmed/clinical – Category: Therapy; Scope: Broad

Diagnostic and Prognostic Studies

https://ncbi.nlm.nih.gov/pubmed/clinical – Category: Therapy; Scope: Broad/Narrow

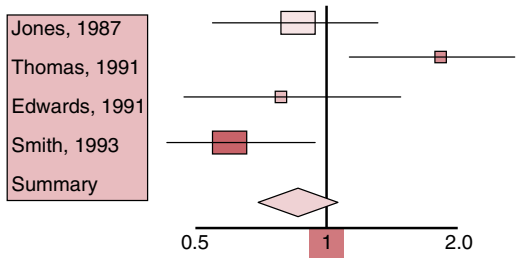
Further Suggestions

- If time is at a premium, look first at sources that synthesise the evidence – for example Transfusion Evidence Library, TRIP Database, BMJ Best Practice (BMJ), ClinicalKey (Elsevier) or UpToDate (Wolters Kluwer).
- Stay up to date by saving your searches and setting up regular alerts in your NLM account in PubMed, or by signing up for the monthly Transfusion Evidence Alert (https://www.transfusionevidencelibrary.com/newsletter).
- Manage your search results by downloading into bibliographic software – for example EndNote, Reference Manager or Zotero – and for research projects, always record the search terms used and the databases and dates searched.
- For further help, or for more comprehensive searching, make a friend of your hospital or university librarian!

Meta-analysis, strictly speaking, means mathematically pooling data from primary studies. This method is acceptable for a systematic review when primary studies are sufficiently homogeneous in their design and quality to show any difference in treatment effect between the two treatment groups. Results from each study within a systematic review are typically presented in the form of a graphical display called a 'forest plot'. A hypothetical example is shown in Figure 48.1.

The result for each outcome point estimate in each trial is represented by a square, together with a horizontal line that corresponds to the 95% confidence intervals (CIs). For summary statistics of binary or dichotomous data, effect measures are typically summarised as either a relative risk or an odds ratio (for definitions, see Figure 48.1). The 95% CI provides a very useful measure of effect, in that it represents the range of values that will contain the true size of treatment effect 95% of the time, should the study be repeated again and again. The solid vertical line corresponds to no effect of treatment (or a relative risk of 1.0 for the analysis of dichotomous data, see Figure 48.1). Forest plots, therefore, are a visual representation of the size of treatment effects between different trials and allow the reader to assess:

- The effect of treatment by examining whether the bounds of the CI exceed or overlap the minimal clinically important benefit.
- The consistency of the direction of the treatment effects across multiple studies.
- Outlying results from some studies relative to others.



- The figure shows a forest plot display for four hypothetical studies.
- The point estimates for each trial have been presented as a relative risk for an outcome with discrete data. The blocks for the point estimates are different sizes, in proportion to the weight that each study takes in the analysis. Weighting is used in order to draw the reader's eye to the more precise studies.
- The relative risk (RR) is the ratio of risk in the intervention group to the risk in the control group. A RR of 1 (RR = 1.0) indicates no difference between comparison groups. For undesirable outcomes an RR that is less than 1 indicates that the intervention was effective in reducing the risk of that outcome.
- The diamond shape represents a summary point estimate for all trials. The vertical line corresponds to no effect of treatment. Thus if the 95% confidence interval crosses the vertical line, this indicates that the difference in effect of intervention therapy compared to control is not statistically significant at the level of $p > 0.05$ (note there will be a 1 in 20 chance that the confidence interval does not include the true value). Such is the case in this example.
- Perhaps, the most important aspect of displaying the results graphically in this way is that it helps the reader look at the overall effects for each trial. Therefore, in this example, it should prompt the reader to ask why the results for one trial seem to be so different from the others (Thomas, 1991).

Figure 48.1 A hypothetical forest plot.

Figure 48.2 provides an overall guide for assessing the validity of evidence for treatment decisions for the different types of studies, trials and reviews mentioned in this section.

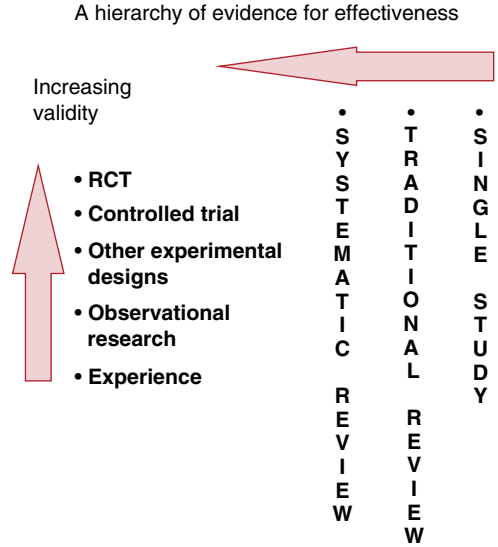


Figure 48.2 A guide for judging the validity of evidence for treatment decisions from different types of studies and reviews. RCT, randomised controlled trial.

Evaluating the Evidence Included in Systematic Reviews

The GRADE (Grading of Recommendations Assessment, Development and Evaluation) tool is a system for evaluating and rating the quality of evidence that is included in a systematic review, although it was first designed to grade the strength of recommendations in guidelines [9]. The GRADE tool focuses on the outcomes examined in a systematic review and involves rating their importance to the clinical setting, evaluating the available evidence according to defined criteria and pulling the evidence together with consideration of the preferences of all stakeholders (patients, society, clinical staff). A GRADE analysis has become an important part of the

systematic review process: although not unanimously used, it is now a requirement of all Cochrane reviews.

Appraisal of Systematic Reviews

Published systematic reviews should be transparent in their reporting of the process used, the primary evidence included and excluded and the final findings of the review, where appropriate reporting in full the findings of any meta-analyses. Guidelines for the reporting of systematic reviews have been developed, including Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) for the reporting of systematic reviews of RCTs and Meta-analysis of Observational Studies in Epidemiology (MOOSE) for the reporting of systematic reviews and meta-analyses of observational studies [10,11]. Quality assessment tools have also been developed for critical appraisal of systematic reviews (CASP; Box 48.1). These appraisals may identify a host of concerns about systematic reviews, including aspects of methodology or the inappropriate use of meta-analysis.

Comparative Effectiveness Research

Comparative effectiveness research (CER) is gaining support from both researchers and funding agencies, particularly in the USA and Canada. CER is defined as the conduct and synthesis of systematic research comparing different interventions and strategies to prevent, diagnose, treat and monitor health conditions. While experimental study designs like RCTs are highly valued methods of CER, they are costly and resource intensive and their results may not be easily generalisable to non-study patients. Non-experimental approaches using observational data are also useful tools for CER, but they are inherently limited by heterogeneous methodologies, diverse designs and susceptibility to bias. As methods of observational studies continue to be refined, the data they derive may become more widely applicable, such as advances in the design of clinical registries and the use of encounter-generated data from sources such as electronic medical records.

The Informing Fresh-versus-Old Red cell Management (INFORM) pilot and definitive trial is an example of CER in transfusion medicine [12,13]. The design was pragmatic: patients were randomised to receive one of two treatments that are already routinely used, thus obviating the need for individual informed consent. These two arms were red cell units that had either been stored for the shortest duration (short-term storage group) or the longest duration. Data were collected in real time from existing electronic databases, thereby reducing costs; and study procedures were streamlined, enabling randomisation of more than 900 patients from a single centre in six months at very low cost. These data will continue to address policy decisions around the maximum storage threshold that would optimise the balance between adequate supply and acceptable risk.

Evidence Base for Transfusion Medicine

So, how good is the evidence base for transfusion medicine? As a first step, identification of all relevant RCTs in transfusion medicine is essential. The UK Blood Services' Transfusion Evidence Library is a comprehensive online database of systematic reviews and RCTs published from 1950 to the present that are relevant to transfusion medicine. The content is identified from comprehensive searches of MEDLINE and transfusion-related conference proceedings and is updated monthly. It also contains a growing number of clinical commentaries on recent important articles in transfusion, in which the findings of the research are discussed within the context of other research, the difference the research could make to clinical practice is explained and any opportunities for further research are highlighted.

Another excellent resource is the Cochrane Collaboration's database of RCTs, the Cochrane Central Register of Controlled

Trials (CENTRAL). This database uses sensitive literature search filters that aim to identify all RCTs that have been catalogued on MEDLINE from 1966 and on the European medical bibliographic database EMBASE from 1980. Other online resources containing collections of high-level evidence for clinicians include the TRIP Database, BMJ Clinical Evidence and PubMed's Clinical Queries. Box 48.2 presents a list of suggested sources that can be searched to identify relevant reports of clinical trials and systematic reviews.

Evidence Base for Transfusion Medicine: Individual Examples

In the following section, we provide further examples of developing the evidence base for the practice of transfusion medicine. These examples have been selected to illustrate different challenges in the reviewing pathway.

Iterative Reviewing

A common topic is defining optimal thresholds for the use of platelet and red cell transfusions, and multiple systematic reviews have been conducted, published and subsequently updated [14–18]. These reviews address one or both of the following questions:

- What is the appropriate threshold haemoglobin level to trigger a red cell transfusion?
- What is the appropriate transfusion policy for prophylactic platelet transfusions?

In the most recent update of the Cochrane review on red cell transfusions, six new eligible RCTs were identified by the authors [18]. When added to the 31 studies in the previous version of this Cochrane review [17], these 6 new RCTs supported a fresh and updated meta-analysis. The updated meta-analysis, which included the recently published TRICS III trial, strengthened the evidence that a restrictive transfusion strategy is safe in patients undergoing cardiac surgery, while continuing to highlight those clinical settings where the evidence base for red cell transfusion was less developed.

Furthermore, the updated review raised the issue of the limitations of using mortality as a primary outcome in clinical trials of red cell transfusion, given that the key results underpinning the safety of restrictive red cell transfusion thresholds have been driven by results for meta-analyses on mortality [18]. In some patient groups, such as those with myelodysplastic syndrome or other chronically transfused outpatient groups, functional outcomes and quality of life may be more relevant for elderly patients than mortality.

With this in mind, in 2019 a group of authors outlined a protocol for a systematic review collating the broad literature on the effects of interventions to treat anaemia on health-related quality of life (HR QoL) and physical function outcomes in patients with myelodysplastic syndromes (PROSPERO 2019 CRD42019125866). As randomised trials alone rarely provide sufficient data on QoL-related outcomes, it was decided to expand the scope of the systematic review to include not only RCTs but also non-randomised controlled trials and observational studies – including case–control, cohort, before-and-after and cross-sectional studies. The source searches were also expanded to include APA PsycInfo® (Ovid®), in order to include literature from the psychological, social, behavioural and health sciences, and CINAHL® (EBSCO®), to cover the nursing and allied health literature. Inevitably, these measures greatly increased the volume of references retrieved at the search stage, and when searches were conducted in May 2020 over 7000 references were identified for the review authors to screen.

An important point illustrated by these multiple reviews is that each update represents a follow-up of a previously completed and published systematic review. Such updates are an important part of EBM, whereby systematic reviews can become

outdated as new primary research is published. Regular (defined as 'as new clinical evidence emerges') updating of a systematic review is critical for keeping it clinically current and usable. Updated reviews add new primary evidence to the data that was published in the previous version(s) of the review.

This iterative process is also well illustrated by the concept of living systematic reviews. A living systematic review is a review that is updated frequently, usually monthly, with each update quickly published online. The usual format of a systematic review applies, and so team and workflow management are critically important. A current example is convalescent plasma as treatment for COVID-19 [19], which is providing a regular synthesis of all the literature, allowing interested blood transfusion services to have a continual record of the combined trial data.

Common Practices of Transfusion and Interventions to Improve Transfusion Practice

Systematic reviews may also be applied to important questions about the evidence base for common or well-established practices in transfusion [20–22]. For example, reviews based on observational, non-randomised studies have addressed the following questions:

- What is the maximum time that one unit of red cells can be out of the fridge before it becomes unsafe?
- How often should blood administration sets be changed while a patient is being transfused?
- Which blood transfusion administration method – one-person or two-person checks – is safer?

It is surprising and salutary to realise that some of these common recommendations appear to be based on only limited evidence, yet are commonly reproduced in guidelines and protocols.

COVID-19 Pandemic

The COVID-19 pandemic created many issues related to timely and iterative literature searching and appraisal. The speed with which new literature started to appear in early 2020, coupled with the dissemination of papers in preprint forms, added to what was, at times, a bewildering amount of data for clinicians to absorb. Two specific clinical scenarios of interest to transfusion medicine were soon identified: what are the most effective anticoagulant strategies and the use of convalescent plasma. Thrombosis was recognised as a major clinical feature of COVID-19 infection early on, yet there were uncertainties regarding the benefits (less thrombosis) and risks (more bleeding) for applying escalating strategies for anticoagulation. All transfusion operators were challenged to reconfigure services to provide convalescent plasma, but the true effectiveness (and indeed cost-effectiveness) of it as a treatment was unknown. Both clinical scenarios as well as many other questions of interest to clinicians and healthcare professionals on the front line required speedy access to the relevant literature.

Box 48.3 provides a framework for the literature approach supported by the Systematic Review Initiative, funded by UK Blood Transfusion Services, during the pandemic. Searches were initially run on a daily basis by an information specialist and databases searched included the following:

- World Health Organisation (WHO) Database of Global Research.
- PubMed.
- Vox Sanguinis International Society for Blood Transfusion (ISBT) Science Series.
- COVID-19 SARS-CoV-2 preprints' subset of medRxiv and bioRxiv (a database of pre-peer-review papers).

The outputs from these searches informed a synthesis report describing the implications of COVID-19 on the transfusion chain from donors, through collection and processing, to patients [23].

Box 48.3 Response to COVID-19 from the UK Blood Service's Systematic Review Initiative.

From January 2020 onwards, research into the COVID-19 pandemic began to produce an ever-increasing amount of published evidence, about which it was vital that all transfusion practitioners were kept informed and up to date. In March 2020, the Systematic Review Initiative (SRI) met as a group to discuss how best to respond to this emergency, and the decision was taken to create a COVID-19 section within the SRI's Transfusion Evidence Library (now freely available worldwide at www.transfusionevidencelibrary.com), to be updated on a weekly basis and sent out to all subscribers as an alert. This process involved every member of the team in the following ways:

- Development of the search strategy: COVID-19 and transfusion/coagulopathy search strategies were devised and tested for PubMed and the WHO COVID-19 Global Literature Database.
- Development of the infrastructure for a new, searchable COVID-19 section on the Transfusion Evidence Library, designed and built in collaboration with Evidentia Publishing.
- Development of the scope, inclusion criteria and subject filters for the new COVID-19 section.
- Searching, download and de-duplicating all transfusion-related literature from PubMed and the WHO database.
- Screening of this large volume of literature (> 50 000 references, as of December 2020) and the application of relevant filters to each included study.
- Uploading of the processed COVID-19 studies to the new section of the Transfusion Evidence Library.

Continuing work involves:

- Weekly searching and screening of PubMed and the WHO COVID-19 database from March 2020 to the present.
- Weekly processing and updating of the Covid-19 section of the Transfusion Evidence Library (pink box in Figure 48.3).
- Weekly (now monthly) transfusion alerts sent out to tens of thousands of subscribers worldwide.
- Monthly searching and screening of the Cochrane Covid-19 Study Register for transfusion-related ongoing trials for inclusion in a *Lancet Haematology* living document [23].

Please visit the Transfusion Evidence Library and subscribe to the Transfusion Evidence Alert: https://www.transfusionevidencelibrary.com/newsletter

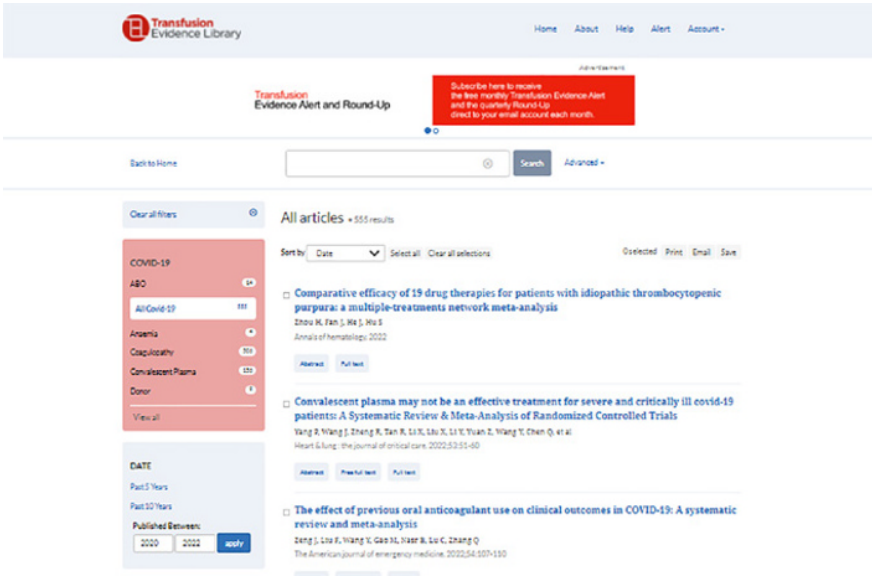


Figure 48.3 Searching the Transfusion Evidence Library.

Conclusion

Systematic reviews and the statistical method of meta-analysis are essential tools to collate and synthesise information but, like trials themselves, can become outdated and must be carefully scrutinised to ensure unbiased results. It is important to acknowledge some of the limitations of EBM that have been discussed by critics and supporters alike. EBM alone cannot provide a clinical decision; instead, the findings generated from EBM are one strand of input driving decision making in clinical practice. Each clinician will also need to consider the available resources and opportunities, the values and needs (physical, psychological and social) of the patient, the local clinical expertise and the costs of the intervention. Patients enrolled in clinical trials are not always the same as the individual patients requiring treatment, and generalising to different clinical settings may not always be appropriate. Transfusion medicine is no different from many other branches of medicine, and the evidence base that informs much of the practice has not developed to the point that it can be universally applied with confidence. There is a need to recognise these uncertainties and to identify those transfusion issues that are high priority for clinical research.

Appraising the evidence base for transfusion medicine is one part of improving practice; another is the effective dissemination of the evidence to clinicians. For example, clinicians may not have the time to search and evaluate the evidence themselves, given the increasing number of publications and journals. As many of the sources are web based, access at any one time has become easier, but easy access needs to be supported by the skills of critical appraisal.

There has been growing recognition that research, especially empirical research (based on observing what has happened), has been underutilised in making healthcare decisions. This appears to be as true for transfusion medicine as for other clinical areas. EBM is an approach to developing and improving skills to identify and apply research evidence to clinical decisions. Even the most ardent proponents of EBM have never claimed it is a panacea, and there is recognition that it should amplify rather than replace clinical skills and knowledge, as well as being a driver for keeping healthcare practices up to date.

Systematic reviews can help bring together relevant literature on a particular problem and assess its strengths, weaknesses and overall meaning. Systematic reviews can be used in different ways, including improving the precision of estimates of effect, generating hypotheses, providing background to new primary research and informing policy. Progress is being made towards ensuring that most areas in transfusion medicine are systematically reviewed and that the resulting evidence informs further research activities and clinical practice.

KEY POINTS

1) The process of evidence-based medicine consists of question formulation, searching for literature, critically appraising studies (identifying strengths and weaknesses) and decisions around applicability to one's patients.
2) It is essential to assess the quality of primary clinical research and consider the risks of evidence being misleading, for example in the case of few trials or a failure to identify appropriate clinical research questions.
3) Systematic reviews of randomised controlled trials combine evidence most likely to provide valid (truthful) answers on particular questions of effectiveness and form an important component in the evaluation of evidence-based practice in transfusion medicine.
4) There is a common perception that much of transfusion medicine practice is based on limited evidence, but this is changing, and systematic reviews are an important tool to collate, analyse and update the evidence base.

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49 A Primer on Biostatistics

Andrew W. Shih[1], *Na Li*[2], *Celina Montemayor*[3] *and Nancy M. Heddle*[4]

[1] *Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada*
[2] *Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada*
[3] *Centre for Innovation, Canadian Blood Services, Toronto, Ontario, Canada*
[4] *Department of Medicine, McMaster University; Canadian Blood Services, Hamilton, Ontario, Canada*

Incidence and Prevalence

There are several measures of disease frequency that are used in the epidemiological and medical literature. Two commonly used terms are *prevalence* and *incidence*. In general, statistics pertaining to *prevalence* are geared towards the question 'How many people have this disease at the moment or during a specific period?' and *incidence* is related to the question 'How many people newly acquire this disease?' Incidence can be calculated as an incidence proportion (typically referred to as risk) or as an incidence rate (typically calculated from cohort studies with long-term follow-up). Definitions of these terms and their calculations are summarised in Figure 49.1.

Statistics in Diagnostic Testing

A perfect diagnostic test would always identify patients as positive if they have the disease and would always be negative in patients without a disease. Unfortunately, the perfect diagnostic test rarely occurs in medical practice. There is typically variation in valid test results for patients both with and without a disease, and a certain degree of overlap between the two.

To help clinicians using diagnostic tests for clinical management, statistics are used to describe the accuracy characteristics of the test, derived from a 2 × 2 table with the test results generally on the columns (positive or negative) and disease/outcome (present or absent) generally on the rows (Table 49.1). Disease status is typically categorised by a test termed the 'gold standard'. Table 49.2 defines the characteristics that describe various aspects of a diagnostic test and their calculations. The terms most commonly used in the literature for diagnostic performance measures are sensitivity and specificity, where a test with a high sensitivity means it is less likely to have a false-negative result and can be used to rule out disease if negative. A test with a high specificity can be used to rule in disease if positive. An example from the literature is demonstrated in Table 49.3.

Statistics used in diagnostic testing can assist clinicians in determining the probability of disease after the results of diagnostic testing are received. The pretest probability

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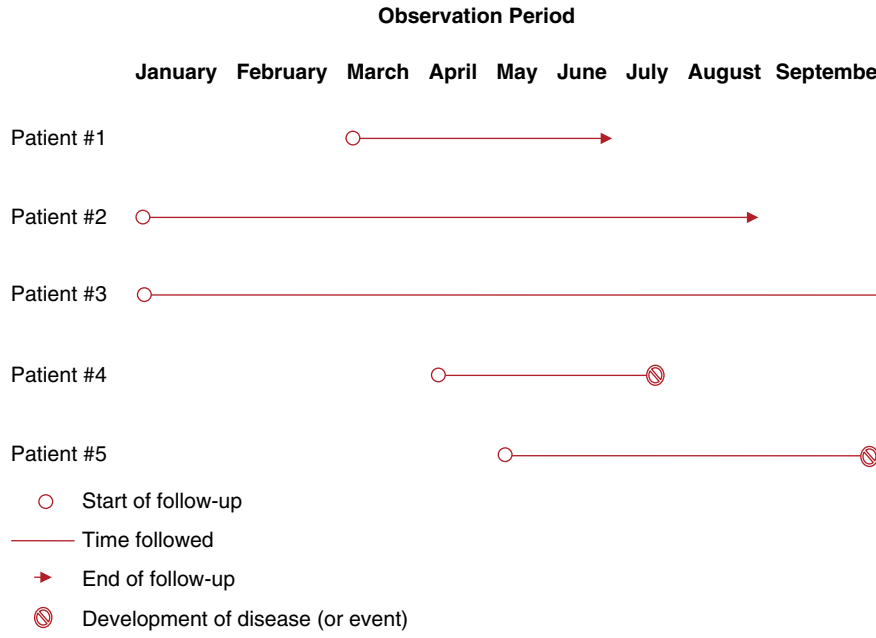

Measure	Formula	Example
Point prevalence	New and existing cases/total population (at a **specific time point**)	Point prevalence in July: 1 case/4 patients = 25%
Period prevalence	New and existing cases/total population (in a **specific period of time**)	Period prevalence from January to September: 2 cases/5 patients = 40%
Incidence proportion	New cases within a time period/total population at risk **within a time period**	Incidence proportion from February to September: 2 case/5 patients = 40%
Incidence rate	New cases within a time period/**total person-time of observation of those at risk**	Incidence rate from January to September: 2 cases/(3 person-months + 4 + 7 + 8 + 4 + 5) = 2 cases/25 person-months

Figure 49.1 Measurements of disease/case frequency.

Table 49.1 Example of a 2 × 2 table for diagnostic tests.

		Disease (often categorised by a test result considered the gold standard)	
		Present	Absent
Test result (New test compared)	Positive	A: True positives	B: False positives
	Negative	C: False negatives	D: True negatives

of having disease is often the prevalence of the disease in the population, if there are no other factors to adjust the pretest probability. With knowledge of the pretest probability, the result of the diagnostic test and the likelihood ratio of that test (defined in Table 49.4),

Table 49.2 Statistical terms used for diagnostic testing.

Descriptive statistic	Definition	Method of calculation
Sensitivity	The proportion of true positives that are correctly identified from those who have the disease. A negative result in a test with high sensitivity is commonly used for ruling out disease (if the test has high sensitivity, the rate of false negatives is low)	True positive/(true positive + false negative) A/(A + C)
Specificity	The proportion of true negatives that are correctly identified from those who do not have the disease. A negative result in a test with high specificity is commonly used for ruling in disease (if the test has high specificity, the rate of false positives is low)	True negative/(true negative + false positive) D/(B + D)
Positive predictive value	The probability that a positive test correctly identifies an individual who has the disease. This value is affected by the prevalence of the disease in the population	True positive/(true positive + false positive) A/(A + B)
Negative predictive value	The probability that a negative test correctly identifies an individual who does not have the disease. This value is affected by the prevalence of the disease in the population	True negative/(true negative + false negative) D/(C + D)
Positive likelihood ratio	The probability that the patient with disease tests positive divided by the probability that the patient without disease tests positive. The higher the positive likelihood ratio, the better the test when positive to rule in disease. Excellent positive likelihood ratios are usually > 10	Sensitivity/(1 – specificity)
Negative likelihood ratio	The probability that the patient with disease tests negative divided by the probability that the patient without disease tests negative. The lower the negative likelihood ratio, the better the test when negative to rule out disease. Excellent negative likelihood ratios are usually < 0.10	(1 – sensitivity)/specificity

the posttest probability of having the disease can be determined. An example of calculating the posttest probability using the Fagan nomogram is given in Figure 49.2 [1].

Descriptive Statistics

Descriptive statistics are used to summarise and describe distributions of data. They are also useful to guide more advanced analyses, which are used to make inferences from the data or to test for differences between groups.

Most statistical programs compute statistics regarding the central tendency of the data as well as some measure of variability within the data [3]. The descriptive statistic that provides the most meaningful summary of the data will depend on whether the data are normally distributed or skewed; hence, it is useful to create a visual display of the data, such as a histogram or a boxplot [4,5]. When data are close to normally distributed, it is appropriate to report the mean and standard deviation. When the data are skewed, the mean may not represent the central tendency

Table 49.3 Example of a 2 × 2 table and diagnostic test characteristics. Erez et al. published a single-centre retrospective study to generate a pregnancy-adjusted disseminated intravascular coagulation (DIC) score, compared to a chart diagnosis of DIC as a gold standard [2]. In 684 women with abruption, 43 had DIC. The investigators used a cut-off score of ≥ 26 to identify pregnant women with DIC and applied it to those with abruption as a sensitivity analysis.

	DIC diagnosis charted in pregnant woman (disease)		
Investigator DIC score ≥ 26 (test result)		Present	Absent
	Positive	A: 38	B: 26
	Negative	C: 5	D: 615
Descriptive statistic	**Method of calculation**	**Calculation from example**	
Sensitivity	True positive/(true positive + false negative) A/(A + C)	38/(38 + 5) = 38/43 = 88.3%	
Specificity	True negative/(true negative + false positive) D/(B + D)	615/(26 + 615) = 615/641 = 95.9%	
Positive predictive value	True positive/(true positive + false positive) A/(A + B)	38/(38 + 26) = 38/64 = 59.3%	
Negative predictive value	True negative/(true negative + false negative) D/(C + D)	615/(5 + 615) = 615/620 = 99.2%	
Positive likelihood ratio	Sensitivity/(1 − specificity)	0.883/(1 − 0.959) = 0.883/0.041 = 21.5	
Negative likelihood ratio	(1 − Sensitivity)/specificity	(1 − 0.883)/0.959 = 0.117/0.959 = 0.122	

of the data, as it is easily affected by extreme observations or outliers [6]. Thus, the median is more appropriate to report along with at least one measure of variability (i.e. interquartile range, minimum/maximum) which provides the reader with more information about the distribution.

Table 49.4 provides the definition of these measures, including an example of how they are calculated. Discussion regarding guidelines for summarising descriptive statistics is outside the scope of this chapter [5].

Differentiating Types of Data and Statistical Tests to be Used

Data analysis will always depend on the research question being addressed, the study hypothesis, the study design and the type of data collected during the study. Thought should always be given to the analysis approach during the design phase of the study. Ideally, each clinical study should have a biostatistician who is a member of the investigative team working with the principal investigator during the planning stages of the study. Bringing in a biostatistician at the end of the study just to do the analysis is typically problematic, and developing a statistical analysis plan should be done as part of the study protocol. Although we identify some considerations for selecting the most appropriate statistical test to use, there are often issues of which most investigators without advanced statistical experience will be unaware.

Considerations for selecting the most appropriate test for analysis are summarised in Table 49.5 and include the following:

- Identifying the dependent and independent variables in a study. The dependent variable(s) is/are the outcome(s) of interest in a study, whereas the independent variable(s) is/are the exposure/intervention(s).

Table 49.4 Descriptive statistics: definitions and example calculation.

Descriptive statistic	Definition	Value derived from example number set: 2, 3, 7, 8, 9, 5, 5, 4, 3, 2
	Measures of the central tendency of the observations in a study sample	
Mean	Calculated by taking the sum of a list of numbers divided by the number of numbers in that list	(2 + 3 + 7 + 8 + 9 + 5 + 5 + 4 + 3 + 2)/10 = 48/10 = 4.8
Median	The number separating the higher half of a data sample from the lower half. The median is more robust than the mean if the distribution is skewed, since the mean is more sensitive to extreme values	2, 2, 3, 3, **4**, **5**, 5, 7, 8, 9 Median = 4.5 (note that in an even set of numbers, it is the average of the middle two values)
Mode	The value that appears most often in a set of data. The mode is infrequently used in medical research	2, 3 and 5 appear twice. Those three numbers are the mode
	Measure of variability of the observations in a study sample	
Standard deviation	Standard deviation measures the dispersion from the mean of a data set. A higher standard deviation indicates that the values are spread out over a wider range. It is often reported together with mean	The sample standard deviation is the square root of $[(2 - 4.8)^2 + (3 - 4.8)^2 + (7 - 4.8)^2 + (8 - 4.8)^2 + (9 - 4.8)^2 + (5 - 4.8)^2 + (5 - 4.8)^2 + (4 - 4.8)^2 + (3 - 4.8)^2 + (2 - 4.8)^2]/(10 - 1)$ = the square root of 6.18 = 2.49
Interquartile range (IQR)	A rank-ordered data set can be divided into four equal parts (quartiles). The values that divide each part are called the 1st, 2nd and 3rd quartiles, denoted by Q1, Q2 and Q3, respectively. The difference between Q3 and Q1 is called the IQR. It is often reported by providing the Q1 and Q3 values	Upper quartile = 7 Lower quartile = 3 IQR = 4 Often reported as IQR 3,7

- Categorisation of the dependent and independent variables by variable type (continuous, categorical, ratio or interval).
- Understanding the distribution of the data.
- Consideration of the study design (i.e. independent groups, before/after, repeated measures, etc.). For randomised controlled trials, the data generally should be analysed by the unit of randomisation (i.e. if patients are randomised then analyse by patient), though there are additional considerations in certain study designs such as cluster randomised trials [7].

Examples of these considerations for data analysis are listed in Table 49.6. After these considerations, selecting an analysis approach that matches the hypothesis is paramount. For this chapter, statistical methods have been categorised as parametric tests, non-parametric tests and regression techniques.

Parametric methods of analysing continuous data assume that the data follows a normal distribution (i.e. a bell curve). These methods are typically not used for data that are skewed. Skewed data can sometimes be log-transformed to create a normal distribution, an approach that should be done if possible [8]. If the data do not follow a normal distribution (i.e. the data are skewed or there is no information about the data distribution), non-parametric approaches can be used. Kaplan–Meier estimates are non-parametric and are used

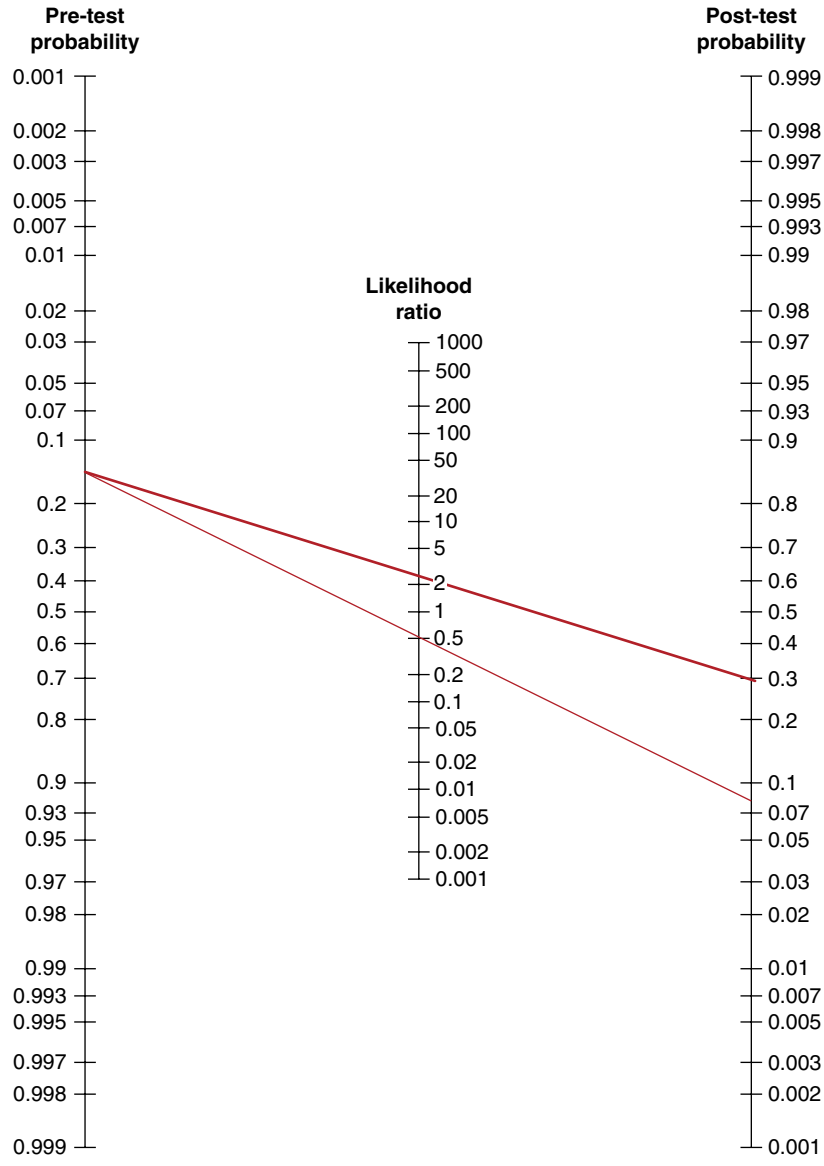

Figure 49.2 Example of the use of a Fagan nomogram. You wish to assess whether a young male seen in your clinic has splenomegaly as a potential reason for thrombocytopenia. Assuming the prevalence of splenomegaly is 3% in the general population, we would normally take the pretest probability as 0.03. However, on history, he has been complaining of early satiety and was referred to you with lymphadenopathy. You assume his pretest probability is higher at 0.15. Now you wish to use percussion of Traube's space (a physical examination manoeuvre) to try to investigate this further. If this test is positive, it has a positive likelihood ratio of 2.21. If this test is negative, it has a negative likelihood ratio of 0.53. The thick line on the nomogram demonstrates the posttest probability if the test were positive and the thin line demonstrates the posttest probability if the test were negative. This patient would have a posttest probability of 0.3 if positive and 0.085 if negative.

for time-to-event analyses, such as doing a survival analysis on the results of a randomised controlled trial. Regression analyses allow the investigator to assess the change in the dependent variable in relation to the change of an independent variable, making them a powerful tool for clinical research. A list and brief description of the more frequently used tests are provided in Table 49.7.

Table 49.5 Considerations for data analysis.

Independent variables			**Dependent variables**	
The input or exposure/intervention (varied by and under the control of the investigator)			The output or outcome of interest (a variable that responds to an intervention or exposure)	
Type of variable				
Quantitative variable types and data measurement scales				
Continuous	**Interval**	**Ratio**	**Ordinal**	**Nominal/ categorical**
Numerical scale that has order and has no discrete limits to gaps between measurements	Numerical scale that has order and intervals between each value are evenly split	Numerical scale that has order and exact value between units, but also has a clear definition of zero	A set of ordered categories	Named categories
Examples				
Weight	Systolic blood pressure	Haemoglobin	Grades of agglutination +, ++, +++, ++++ WHO Bleeding Scale (measured from Grade 1 to 4, with 4 as the most severe)	Dead/alive
Data dependency*	**Independent data/samples/observations**		**Dependent data/samples/observations†**	
Definition	When data, samples or observations are obtained from different subjects and/or samples are not dependent on each other, the data are said to be independent		When data, samples or observations are obtained by repeated measures from the same individual (i.e. before and after an intervention/exposure), the data are said to be dependent	
Example	An observational study of acute reactions in 20 patients receiving their first platelet transfusions. Each patient is transfused platelets and followed for evidence of a reaction following only their first transfusions. Each observation (reaction yes/no) comes from an individual patient, thus the data are independent		An observational study of acute reactions in 20 patients receiving platelet transfusions, in which 10 patients are followed during two different transfusion episodes; the other 10 only receive one transfusion. Data are dependent, as there are 30 observations with 10 patients contributing two different measurements	

* Not to be confused with the dependent/independent variables described above.
† Requires additional considerations during analysis to account for the fact that observations in some patients may be related or dependent on each other.

Table 49.6 Examples of considerations for data analysis.

Study	ABLE Study: Multicentre RCT comparing ICU patients receiving fresh blood (age < 8 days) to patients receiving the oldest available blood, with 90-day mortality as the primary outcome (NEJM 2015;372:1410–18)	MIRACLE Study: Multicentre RCT comparing patients with chemotherapy-induced thrombocytopenia receiving pathogen-reduced platelets to patients receiving standard platelet products, with the 1-hour corrected count increment (CCI) as the primary outcome (Transfusion 2010;50:2362–75)
Independent variables	Transfusion of fresh or older blood – categorical variable Age (days) is an interval variable, but was used as a categorical variable for the intervention in this study	The transfusion of pathogen-reduced platelets or standard platelet products – categorical variable
Dependent variables	90-day mortality – categorical variable (dead or alive) Length of hospital stay (days; secondary outcome) – ratio variable (arguably is non-zero)	1-hour CCI – interval variable Bleeding defined by WHO Bleeding Scale (secondary outcome) – ordinal variable
Data dependency	Independent – patients in either transfusion group (fresh or older blood) do not affect each other; each patient only contributes one outcome as well (assuming that patients are studied during just one hospital admission)	Dependent – because different patients had different (and multiple) episodes of platelet transfusions, each patient would contribute a different number of events; one patient contributing six events is not the same as three patients contributing two events, requiring a specialised analysis approach to deal with the dependency

ICU, intensive care unit; RCT, randomised controlled trial; WHO, World Health Organization.

Determining Statistical Significance

Confidence intervals and p values are often used to determine whether a test result is statistically significant. However, an understanding of all the following terms is important not only for interpretation of results, but also for sample-size calculation:

- Type 1 error (or α error) is the probability of a false-positive conclusion by chance, and is usually set at 0.05. In other words, it is the probability of concluding an effect when the effect is not present.
- Type 2 error (or β error) is the probability of a false-negative conclusion by chance – the probability of not detecting an effect when it is present. This value is typically set at 0.1–0.2.
- The power of the study to detect an effect if it does exist is defined as 1 – β error. Hence, a β error of 0.1 will result in 90% power and 0.2 will have 80% power.
- The p value is a probability (between 0 and 1) of getting the observed value of the test statistic or a value with even greater evidence against the null hypothesis, if the null hypothesis is actually true. The smaller the p value, the greater the evidence against the null hypothesis. A relatively simple way to interpret a p value is to think of it as representing how likely it is that a false-positive conclusion (falsely rejecting the null hypothesis) would occur by chance. A p value of less than 0.05 is typically used as the cut-point to reject a null hypothesis [9].

A confidence interval (CI) is a plausible range of values for a population parameter. A 95%

Table 49.7 Commonly used parametric, non-parametric, categorical and time-to-event statistics.

	Application	Example	Example reference
Tests for parametric data			
t-test	Compares continuous outcomes (interval or ratio) in two independent samples	A study comparing quantitative antibody levels after vaccination between immune thrombocytopenic purpura (ITP) patients receiving rituximab versus placebo	Blood 2013;**122**:1946–53
Paired t-test	Compare continuous outcomes in two matched or paired samples	A study comparing platelet counts in ITP patients before romiplostim and after romiplostim was started (assuming that the values of the differences are a normal distribution)	Transfusion 2016;**56**(1):73–9
One-way analysis of variance (ANOVA)	Comparison of two or more groups where the independent variable is interval or continuous	A study assessing pulmonary hypertension in sickle cell disease patients chronically transfused, non-transfused sickle cell disease patients and age-matched controls (assuming the outcome is in a normal distribution)	Blood 2015;**126**:703–10
Repeated-measures ANOVA	Used for analysis of repeated-measure study designs. The test requires one categorical independent variable (either nominal or ordinal) and one dependent variable (continuous: interval or ratio)	A study assessing FiO_2 (fraction of inspired oxygen) levels measured repeatedly in patients before and after transfusion who have transfusion-related acute lung injury (TRALI) or do not have TRALI	J Perinatol 2013;**33**:292–6
Factorial ANOVA	Used to consider the effect of more than one factor on differences in the dependent variable	A study assessing nitric oxide levels in blood samples with different durations of storage of red cells and different pO_2 (partial pressure of oxygen) levels set by the investigators	Biochem J 2012;**446**:499–508
Simple linear regression	Analysis of data with one independent variable and one or more dependent variables that are interval. This analysis assumes that the relationship between the dependent variables and the independent variable is linear	A study assessing in intensive care unit (ICU) patients after red cell transfusion the association between severity of illness at ICU admission (via the APACHE II score) and nadir haemoglobin on the day of red cell transfusion	Crit Care Med 2009;**37**:1935–40

(*Continued*)

Table 49.7 (Continued)

	Application	Example	Example reference
Multiple linear regression	Analysis data with one dependent variable and several independent variables (interval-level data)	A study assessing how different variables (such as platelet age and patient body surface area) affect posttransfusion absolute count increments for platelet counts in ABO-compatible and ABO-incompatible platelet transfusions	Transfusion 2010;**50**:1552–60
Logistic regression	Used when the dependent variable is binary. This analysis assumes that the relationship between the log odds of the dependent variable and the independent variable is linear. Multiple independent variables may also be included in a multiple logistic regression test	A study assessing 90-day all-cause mortality in ICU patients receiving fresh (< 8 days) compared to those receiving the oldest available compatible blood, after adjusting for confounding variables such as age, sex, illness severity and co-existing illnesses	NEJM 2015;**372**:1410–18
ANCOVA	Combines ANOVA and regression (example: can be used to assess treatment effects while controlling for baseline characteristics)	A study comparing plasma volume used in patients receiving plasma prepared with pathogen inactivation and conventional plasma, while adjusting for co-variates such as patient demographics or model end-stage liver disease (MELD) score	Transfusion 2015;**55**:1710–20
Tests for non-parametric data			
Mann–Whitney U test (Wilcoxon rank sum)	To compare a continuous outcome in two independent samples given the data is ordinal or in a non-parametric distribution	A study comparing the plasma to red cell ratio in massive haemorrhage patients who survived the protocol and those who did not survive the protocol (the distribution of plasma: red cell ratios is expected to be skewed)	J Trauma 2007;**63**:805–13
Kruskal Wallis test	To compare a continuous outcome in more than two independent samples	A study assessing pulmonary hypertension in sickle cell disease patients chronically transfused, non-transfused sickle cell disease patients and age-matched controls (assuming the outcome is not in a normal distribution)	Blood 2015;**126**:703–10
Wilcoxon sign rank test	To compare a continuous outcome from two related samples, matched samples or repeated measurements on a single sample to assess whether their population mean ranks differ	A study comparing intravenous immunoglobulin (IvIg) utilisation in ITP patients before romiplostim and after romiplostim was started (assuming that the values of the differences do not meet a parametric distribution)	Transfusion 2016;**56**(1):73–9

Tests for categorical data			
Chi-square test	To test for significance using categorical frequency data	A study comparing successful immunity after vaccination between ITP patients receiving rituximab versus placebo (provided there are at least five events in each cell of a 2 × 2 table)	Blood 2013;**122**:1946–53
Fisher exact test	To test for significance using categorical frequency data when the numbers in one or more of the cells in the 2 × 2 table are less than or equal to five	A study comparing successful immunity after vaccination between ITP patients receiving rituximab versus placebo (provided there are five or fewer events in each cell of a 2 × 2 table)	Blood 2013;**122**:1946–53
McNemar test	Used with categorical frequency data when observations are paired/matched	A study comparing seroconversion rates for heparin-induced thrombocytopenia (HIT) antibodies in orthopaedic patients receiving fondaparinux and enoxaparin in separate episodes	Blood 2005;**106**:3791–6
Tests for time-to-event data			
Survival analysis (i.e. Kaplan–Meier method and logrank test)	Allows an analysis of how long people are in one state (i.e. alive) followed by a discrete outcome (death). People can enter the study at different times and are followed for variable periods of time. The Kaplan–Meier curve is a common method of visualising the probability of the discrete outcome at different time intervals. Two or more groups can be compared, with the logrank rest used to test the null hypothesis (determining whether there is a statistically significant difference)	A study following patients after receiving a massive haemorrhage protocol with a 1 : 1 : 1 ratio compared to a 1 : 1 : 2 ratio up to 24 hours and 30 days	JAMA 2015;**313**:471–82
Cox regression (or proportional hazards regression)	Used to determine the relationship of several risk factors and/or exposures to survival time. This produces a hazard rate as the measure of the event risk up to a specific time point	A study assessing in-hospital mortality in hospitalised patients receiving red blood cell transfusions that had been stored for the shortest duration compared to the longest duration. In a secondary analysis, the maximum storage duration in days of transfused red cells was used as a time-dependent co-variate in a Cox regression model, stratified by study centre, blood type and the cumulative number of units received	NEJM 2016;**375**:1937–45

CI means that 95% of the intervals constructed would cover the true population parameter. Another way of expressing this is that if the study were repeated on multiple samples (say 100 samples), 95 of the calculated CI (which would likely differ for each sample) from the 100 samples would encompass the true population parameter. The CI provides more information than a p value, as it shows the uncertainty we have around the point estimate.

Multiple Tests of Significance

A common mistake seen in some publications is the use of multiple tests of significance using the same data. As stated previously, if we took a set of data and performed a single test of significance with the type 1 error set at 0.05, the probability of obtaining a significant result by chance would be 5%, or 1 in every 20 tests performed. If three tests of significance are done and each uses a p value of 0.05, now the probability of obtaining at least one statistically significant test result by chance would be 14%, calculated as $1 - (1 - 0.05)$ [4]. This reflects the higher probability of a false-positive finding if more tests of significance are used on the same data set.

This mistake is frequently seen in baseline characteristic data reported from a randomised controlled trial where many different variables are compared with individual tests of significance. In this example, randomisation should have provided balance between the two treatment groups and if the test on a variable is statistically significant, it would simply be due to chance.

To deal with multiple tests of significance, statistical approaches that adjust the p value based on the number of tests performed are used. The Bonferroni correction is one of the most commonly used methods, where the desired level of significance of the group of tests is divided by the number of hypotheses being tested. This correction can be conservative and produce false-negative results. Other methods include Tukey's honestly significant difference and Scheffe's test.

Trial Hypotheses and Common Pitfalls of Interpretation

A well-defined research question using the PICOT format (Patient, Intervention, Comparison, Outcome and Time) is necessary for the proper design of a clinical study [10]. The question should clearly state or imply the hypothesis for the study. In clinical research there are three possible hypotheses: superiority, non-inferiority and equivalence. Each of these hypotheses requires different sample-size calculations, different approaches to analyses and interpretation that is specific to the hypothesis being studied [11].

Superiority Trial

Superiority trials are usually designed to determine if a new treatment is better than an active control (such as current standard of care) or placebo. Thus, they attempt to prove the hypothesis that the intervention will have Δ_e (the true difference in effect) over the control group. This is the most common type of trial reported in the medical literature.

The analysis for the primary outcome for a superiority study should be an intention-to-treat analysis, where groups are analysed based on the initial treatment assignment and not on those who comply with the study protocol (the latter is termed a 'per-protocol' analysis, typically including only those who receive the treatment they are assigned to). This type of analysis reflects what occurs in a 'real-world' setting where there are likely to be co-interventions, crossovers and dropouts in trials. For example, the effect of a placebo-controlled trial of a new drug with many side effects would likely be overestimated with a per-protocol analysis compared to an intention-to-treat analysis.

When performing a superiority trial, the null hypothesis is that the effect of the treatment is not different from the control group. If the p value of the analysis is significant (≤ 0.05), the null hypothesis can be rejected and superiority claimed. One of the most

common pitfalls when interpreting a negative superiority trial ($p > 0.05$) is a conclusion of equivalence or non-inferiority that is not valid.

Equivalence Trial

Equivalence trials are designed to show that two treatments are equal within an acceptable defined boundary (referred to as the margin of equivalence). It is statistically impossible to demonstrate that two treatments have identical efficacy. Therefore, the design of an equivalence trial would demonstrate that the observed effect difference between an intervention and the control, as indicated by the width of the CI, should not be outside a boundary of $-\Delta_e$ and Δ_e (the true difference in a negative or positive direction, respectively). The null hypothesis in an equivalence study is that the treatment effect is not similar, so a significant p value (≤ 0.05) allows for rejection of the null hypothesis and a conclusion of equivalence.

Non-inferiority Trial

Non-inferiority trials are often used to demonstrate that the effect of an intervention is not inferior to the comparison treatment, usually with the assumption that the intervention has other ancillary benefits (such as fewer side effects, ease of administration or monitoring or decreased cost). The null hypothesis in a non-inferiority trial is that the experimental treatment being compared is inferior to the control. If the p value of the analysis is ≤ 0.05, the null hypothesis can be rejected and non-inferiority claimed.

In a non-inferiority trial, the boundary for establishing non-inferiority (zone of non-inferiority) is a clinical decision as to what physicians are prepared to accept as a trade-off between risk and benefit [12]. The sample size for a non-inferiority study should generally be calculated with a relative risk difference rather than an absolute risk difference, as this gives a more conservative sample size. A new intervention should ideally be compared to the current or most effective standard of care, or a phenomenon known as 'biocreep' can occur. When a slightly inferior treatment becomes the active control for future generations of trials, this may lead to the efficacy of the intervention getting worse with repeated cycles. This phenomenon has been described by Murphy in relation to platelet product efficacy [13].

Finally, both intention-to-treat and per-protocol analyses should be included in a non-inferiority study. While intention-to-treat analyses in a poorly run superiority trial with unintended crossover, loss to follow-up and non-adherence will produce a negative result, intention-to-treat analyses in a poorly run non-inferiority trial will tend to favour a 'positive' result of non-inferiority. Results in this situation require careful consideration.

Meta-analyses and Forest Plots

Meta-analyses

A systematic review is a focused literature review on a research question, where articles are identified and selected, and information synthesised together to provide the best summary of the literature available. The search strategy for the literature, the eligibility criteria for inclusion in the review and the methodology for data extraction and assessment of the articles are defined *a priori* before the search begins.

If studies are similar enough in nature in terms of clinical and methodological characteristics and the risk of publication bias is minimal, a statistical summary called a meta-analysis can estimate a pooled 'effect size' of several studies. Typically, it estimates a 'weighted' average across all the included studies. This can aid in summarising literature, resolving conflict in an area of medical therapy, increasing statistical power and identifying gaps in the literature.

The correct statistical model should be determined for the meta-analysis. A comparison of fixed- and random-effects models is shown in Table 49.8. If it is possible, both

Table 49.8 Comparison of fixed- and random-effects models in meta-analyses.

	Fixed-effects models	Random-effects models
Assumptions	Assumes there is one true effect size of the intervention across studies	Assumes the true effects of the intervention can differ across individual studies, so the goal is to estimate the mean of a distribution of the true effect sizes
Analysing methods	Mantel–Haenszel method	DerSimonian–Laird method Consider Knapp–Hartung correction or profile likelihood approach to adjust confidence intervals to avoid type 1 error
Type of model with the inverse variance method	When variance is within studies	When variance is between studies and within studies
Characteristics	Produces a more accurate and precise result in studies based on the same study population. Not applicable in fields where there is heterogeneity between studies	Likely more applicable in medical literature given studies based on universe of study populations. The relative weights assigned under a random-effects model will be more balanced than those assigned under a fixed-effects model. Extreme studies will lose influence if they are large, and will gain influence if they are small. It is hard to meet the model assumptions: it is hard to prove the study populations in the studies were from a universe of populations; it instead assumes a random sample from the population, which is not usually the case for observational studies; it requires a larger sample size of studies to more accurately estimate the between-study variations

fixed- and random-effects models can be used and compared [14].

Forest Plots

Forest plots are graphs that summarise the individual study results in a systematic review and the pooled effect if the results are meta-analysed [15]. Generally, point estimates of each study are shown on the right of the forest plot around the vertical 'line of no difference.' Although the literature commonly formats point estimates to the left of the line of no difference as favouring the intervention, occasionally this is reversed. An example of a forest plot is given in Figure 49.3.

Often, below the numerical pooled estimate, the consistency between results of the included studies is reported. Heterogeneity can be assessed in different ways. If the chi-square statistic is greater than the number of studies minus one, then there is heterogeneity outside what would be expected by chance. Cochrane's Q Test or I^2 is generally considered a more robust test, given that the chi-square is not robust if studies have very small or large sample sizes. The higher the I^2 statistic, the more heterogeneity, and if this is greater than 50% it indicates that the validity of the overall estimate may be in question. An imaginary vertical line from the point pooled estimate intersecting all the 95% CIs can also be used as a rough estimate that the summary statistic is consistent with the results of the included studies [16].

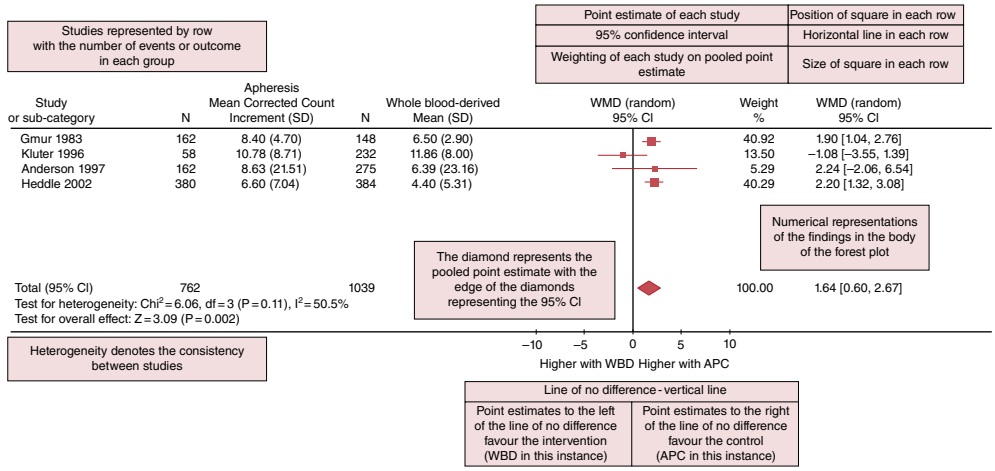

Figure 49.3 Example of a forest plot. Meta-analysis of the weighted mean difference in the 1-hour corrected count increments of whole blood–derived (WBD) platelets compared to apheresis platelet concentrates (APC). CI, confidence interval; SD, standard deviation; WMD, weighted mean difference. *Source*: Adapted from Heddle NM et al. Transfusion 2008;**48**:1447–58.

Emerging Data Science Approaches: Predictive Analysis in Transfusion Medicine

While traditional methods use a test and/or a diagnostic tool to predict a disease state and/or outcome, as discussed at the beginning of this chapter, predictive analysis is becoming increasingly popular due to the emergence of sophisticated data science. That is, aiming to predict future events or outcomes based on evidence from historical and current data [17], assuming the future will not be dramatically different from both the past and the present for the area of study. Accurate predictive models can help guide decisions on diagnosis and/or treatment.

A key difference between predictive analytics and traditional exploratory methods is that predictive analytics apply statistical models and/or data mining techniques, without a prior hypothesis, using predictors that may not be causal factors for the outcome of interest [18]. To understand the difference, let us compare two studies. (1) Consistent with a traditional exploratory method, a cross-sectional study using pretested, self-administered, mailed questionnaires was designed to assess factors that could be associated with perioperative transfusion decisions in patients with coronary artery disease [19]. The study selected a set of potential factors that may affect physicians' decisions (based on a literature search), and then evaluated the associations and effect sizes of those factors, using a mixed-effects regression model. (2) Consistent with predictive analytics, a recent study used a large surgical database to develop and validate an artificial neural network (ANN) model for perioperative transfusion prediction [20]. The large data set provided the ANN with the ability to 'learn' by processing examples of inputs and outcomes from a training data set, which then formed probability-weighted associations between factors and perioperative transfusion. The accuracy of the ANN model was then assessed using a separate test data set. The sensitivity, specificity and area under the curve (AUC) of predictors to the studied outcome were then extracted from the validated model. This method has the advantage of finding potential associations that are not necessarily identified *a priori*. Although these methodologies have similarities, their objectives are distinct, leading to different interpretations and uses of knowledge. Poor understanding of the differences between explanatory methods and predictive research can lead to low-quality research and misleading conclusions [18].

Emerging Data Science Approaches: An Introduction to 'Big Data'

The term 'big data' has gained increasing popularity in contemporary medical and scientific literature. Although there is no formal quantitative definition for this category, big data is generally conceived as a collection of information that expands across the 3Vs: volume, velocity and variety [21]. Simply stated, big data science refers to the analysis and interpretation of data sets of extraordinary size, produced at an equally extraordinary speed, and often consisting of disparate data types and formats. Common players within the healthcare arena include electronic medical records, diagnostic imaging, mobile biometric sensors, 'omics' data (genomics, proteomics, metabolomics), environmental records and public health figures [22,23]. Frequently, integration of multiple big data sources is sought for descriptive, predictive, prescriptive and/or preventive purposes. Examples of these studies include the All of US programme and the Recipient Epidemiology and Donor Evaluation Study (REDS) programme.

Big data provide powerful advantages and opportunities, but also present unique data handling and analytical challenges. As conventional hardware and software tools are often inadequate, novel analytical and

visualisation strategies that strive for accurate interpretation within a reasonable timeframe have been developed [23]. Intimately linked to computational science and infrastructure, these approaches often involve parallel data processing across multiple computational nodes, along with establishment of robust 'big data management programmes'. Such programmes must address data security, integrity, storage, backup, provenance and documentation elements. Discussion of specific analytical tools is beyond the scope of this chapter; big data strategies are continuously evolving, but always centre around the principles of reproducibility, interoperability and security [22,24]. The need for a skilled bioinformatics team to collaborate from the inception of the study cannot be overstated.

Within the big data disciplines, genomics deserves special attention due to its decreasing cost, exponentially increasing data acquisition volume and rate, and potential to tailor medical treatment, diagnostic and prevention strategies [25]. Within clinical genomics, blood antigen prediction has been proposed as a potential future universal application [26]. Applicable processing algorithms are in continuous development, often adapted for specific circumstances, and are typically assembled into a customised analytical sequence or 'bioinformatic pipeline' [26]. Careful design and validation of these pipelines are paramount to ensure the accuracy of the results and are mandatory in the clinical setting [27].

Conclusion

While we have only scratched the surface of biostatistics with this chapter, the concepts discussed provide a framework for the interpretation of studies commonly seen in the medical literature. When performing research, consulting with a biostatistician is paramount to ensure that the study is designed in a way that facilitates proper analysis. However, having knowledge of the concepts in biostatistics ensures that the clinical goals of the study meet the study design and the analytical plan.

KEY POINTS

1) When using descriptive statistics, the mean is generally used when data follow a normal distribution. When data are skewed, the median and interquartile ranges are a better representation.
2) In diagnostic testing, a negative test that has a high sensitivity helps to rule out disease. A positive test that has a high specificity helps to rule in disease.
3) The higher the positive likelihood ratio, the better the test when positive to rule in disease. The lower the negative likelihood ratio, the better the test when negative to rule out disease.
4) The best statistical test to determine whether there is a relationship between independent and dependent variables depends on the variable type, the independence of the data and the number of variables.
5) Type 1 error is the probability of a false-positive result. When reporting results, consider using 95% confidence intervals rather than the p value. Type 2 error is the probability of a false-negative result. When considering sample-size calculation for a new study, it is recommended to set the type 1 error to 0.05 or lower and the type 2 error to 0.2 or lower (this corresponds to the power of the study to 80% or higher).
6) If multiple tests of significance are done, statistical approaches to adjust the p value are necessary.
7) A negative finding in a superiority study does not mean equivalence or non-inferiority.
8) The following are optimal for the design of a non-inferiority study: setting sample size based on relative risk difference, use of an active control that is the most effective

standard of care and including a per-protocol analysis.

9) Studies should not be meta-analysed when the clinical diversity is too great, there is a significant risk of publication bias or methodological diversity is too great.

10) Analytical techniques using larger data sets can be helpful for predictive analyses as well as tailoring medical interventions. Such 'big data' approaches require appropriate infrastructure, and above all collaborators with highly specialised biostatistics and bioinformatics skill sets.

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50 A Primer on Health Economics

Seema Kacker and Aaron A. R. Tobian

The Johns Hopkins University School of Medicine and Bloomberg School of Public Health, Baltimore, MD, USA

Health spending continues to rise, both domestically and globally; in the USA, national health expenditure is expected to grow by an average of 5.4% per year between 2019 and 2028, with healthcare costs as a portion of gross domestic product expected to reach 19.7% by 2028 [1,2]. Costs within the field of transfusion medicine have followed these trends [3–5], due in part to increased demand for transfusion services, increased utilisation of risk-reduction methods to improve blood supply safety and the implementation of new technologies in donation and transfusion [6]. Blood remains a scarce resource and, as such, consideration of the relevant costs and financial implications of transfusion practices and policy is warranted.

Blood transfusion costs may seem negligible when considering the overall cost of healthcare – transfusion represents just 1% or less of total costs for most conditions [7]. However, the proportion of total hospital costs attributable to blood transfusion varies greatly by disease and procedure. For some treatments, including liver and bone marrow transplantation, transfusion plays a more substantial financial role; the cost of blood products alone for these treatments can exceed $3800 (equivalent to £2300), or 5–9% of total hospital costs [7]. Transfusion-associated complications can also result in costly hospital stays and treatments [8,9].

As our population ages and we increasingly use procedures and treatments that require transfusion, the demand for blood products will likely be sustained. These trends and the associated expenditures raise concerns about sustainability and value in our healthcare system, and have prompted increased attention to the field of health economics. Health economics is concerned with effectiveness, efficiency and behaviour as they relate to the allocation of health and healthcare, and uses rigorous analytical methods to understand the behaviour of the many players within the healthcare system – patients, providers, public and private payers, communities, etc. It addresses questions such as: Is technique A or B more cost-effective? Or, how have various donor policies or donation campaigns affected the supply of blood and blood products? Or, in a context of a limited blood supply, what resource allocation strategies would help to maximise total welfare? Or even, what is the overall economic impact of transfusion-transmitted human immunodeficiency virus (HIV) infection or implementation of pathogen-reduction technology? Using an economic lens to understand issues

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in transfusion medicine can have important implications for policy design and, ultimately, for patient care.

How Economists Think about the Blood Supply and Transfusions

Economists think about how to allocate a limited set of resources given some consumer demand. Health economists can view health as a stock variable – individuals have some initial endowment of 'health' and, in the absence of investments like nutrition and physical activity and medical care, the stock of 'health' declines over time. In this set-up, 'health' leads to some level of happiness, or utility, and individuals choose to 'purchase' some amount of medical care to improve their overall utility, given a set of preferences and trade-offs [10]. Of course, in the actual healthcare market it is difficult to simply 'purchase' an amount of healthcare to keep up stocks of health and happiness. Health and healthcare are complex because the market for healthcare is not one where individuals receiving care are fully responsible for paying for it. Furthermore, individuals receiving care often do not know exactly what they are getting, and another agent – a physician, for example – is often largely responsible for determining the kind and quantity of care provided. Still, the framework of understanding health as being produced by a number of factors, including healthcare, can be useful.

Supply and Demand for Blood

Although blood is usually donated for free by altruistic individuals, there are significant costs associated with its collection, testing, component preparation and labelling, storage, shipping and transfusion. These costs are often borne by blood centres and hospitals, and passed on to patients, providers and insurance firms, although these arrangements may vary and may be quite complicated. From an economic perspective, donors also experience costs in terms of any direct or indirect expenses they bear in the donation process and the opportunity cost of their time.

There have been slight declines in the prices hospitals pay for blood products in recent years; the 2017 National Blood Collection and Utilization survey in the USA found that the median price paid for blood components by hospitals decreased from $211 to $207 for leuco-reduced red blood cell (RBC) units between 2015 and 2017. Declines of similar magnitude were seen for apheresis platelets and fresh frozen plasma [11].

'Processing fees' vary substantially across different blood products, as well as across different geographical regions and facilities. A costing analysis of four hospitals transfusing surgical patients found that the cost of transfusing a single red cell unit ranged from $522 to $1183 (£344 to £766) [5]. This variation can be partly explained by differences in the efficiency and scale of processing and other overhead charges. Additional processing and laboratory testing, as well as decreases in the donor pool, can also increase the overall cost of transfusion [5,12].

The demand for blood products in the USA has declined over recent years; a study using the Nationwide Inpatient sample showed a 17% decrease in RBC transfusion between 2011 and 2014 [13]. This downward trend has continued through 2018. This decrease may be attributable to increased use of patient blood management programmes to reduce unnecessary transfusion and associated risk [14], as well as an increased use of surgical techniques and methods that minimise blood loss. A blood management programme at Stanford Hospital using real-time electronic best practices alerts resulted in a 24% reduction in total red cell transfusions and a reduction in the number of patients transfused outside haemoglobin trigger guidelines by nearly 50%. This led to savings of $1.6 million (£1 million) in purchase costs

per year [15]. Similar results have been reported elsewhere [16,17]. Decreased transfusion could, of course, also be beneficial by reducing the risk of transfusion-transmitted infections and other associated complications. It is important to note that these data do not account for changes in demand as a result of COVID-19.

Maintaining a sufficient blood supply can be challenging; the blood available for use in a hospital is dependent on a number of factors, including the rate of donation, the way blood products are allocated across regions and hospitals, and blood product shelf-life and storage requirements. In the USA, just 3% of age-eligible people donate blood every year [18]. Similarly, NHS Blood and Transplant (NHSBT) in England estimates that only 4% of the eligible population donates blood [19]. In this context, blood collection organisations must hold frequent campaigns to recruit donors. Donation rates may also fluctuate unexpectedly; many blood drives have been cancelled due to COVID-19, leading to critically low supplies in some settings [20].

In the USA, the overwhelming majority of blood donation is voluntary; while donors may be given small gifts (e.g. T-shirts, pens, cookies) for their donation, they are generally *not* financially compensated. This practice does vary globally, however, with higher-income countries relying more often on voluntary whole-blood donations compared to lower-income countries [21].

From an economics lens, a volunteer-dependent blood supply can result in the problem of 'free-riding': anyone can benefit from its use as a 'common good', but only some individuals will choose to contribute to it. There is ongoing discussion about the potential role of financial or other extrinsic incentives in increasing blood donation, although some commentators have raised potential ethical and safety concerns [21]. Interestingly, the overwhelming majority of plasma that is donated in the USA is financially compensated, with donors often paid $30–60 per donation [21].

Economic Evaluation in Transfusion Medicine

Design

The field of transfusion medicine has increasingly relied on economic evaluation in the past several years to help clinicians and policymakers maximise efficiency and patient benefit while minimising cost [22]. These analyses are particularly relevant for evaluating risk-reduction methods in blood collection and processing as well as in transfusion, but also for evaluating the patient, hospital and social impact of new policies and management strategies.

Multiple forms of economic evaluation exist, including cost analyses, cost-minimisation analyses, cost-effectiveness analyses, cost–utility analyses and cost–benefit analyses. Each of these will be described briefly, including relevant examples, to provide a general understanding of how to understand and interpret these types of analyses. A summary is provided in Table 50.1. Further guidance on how to perform these economic evaluations is detailed elsewhere [22–26].

Cost analyses assess the resources being used for a given programme or policy. For example, a blood supplier might be interested in evaluating the overall costs associated with a donation campaign and a subsequent blood drive held in a community centre. This type of analysis might consider costs associated with designing and producing advertisements, training, salaries or compensation for individuals involved in the campaign, rental fees for the community centre space, equipment and supplies necessary for the drive and for donors, salaries for nurses and technicians, waste management, product testing and processing.

While many studies focus on direct medical expenses only, others incorporate a broader set of costs, also including direct non-medical expenses and intangible and productivity-related costs. Direct medical expenses include the costs of goods, services or other resources consumed for the

Table 50.1 Summary of economic evaluation designs.

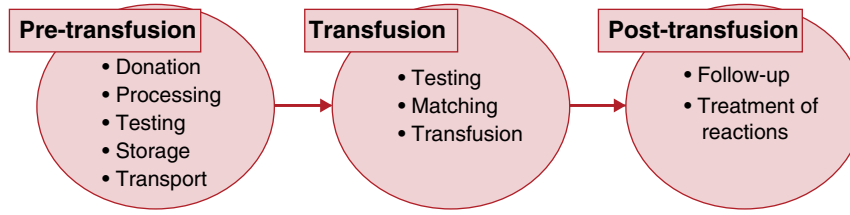
Type of economic evaluation	Outcomes	Example	Notes
Cost analysis	Costs ($)	Lifetime cost of chronic transfusion for patient with sickle cell disease	Cost discounting may be important for long-term analyses
Cost minimisation	Costs ($)	Comparison of cost for hospital to store emergency blood in operating rooms or in blood transfusion laboratory	Similar to cost analysis
Cost-effectiveness	Costs ($) Effectiveness (natural units – infections averted, lives saved, cases identified, etc.)	Financial and health impact of incorporating HIV nucleic acid testing in the blood supply	Estimate cost-effectiveness ratio (average or incremental) Effectiveness discounting
Cost–utility	Costs ($) Utility (QALYs, DALYs)	Cost–utility analysis of an electronic medical record check to confirm that a transfusion is appropriate for a particular patient	Similar to cost-effectiveness but using a standard effectiveness measure
Cost–benefit	Costs ($) Benefits ($)	Cost–benefit analysis of alternative blood donation campaigns	Monetises health outcomes

DALY, disability-adjusted life-year; HIV, human immunodeficiency virus; QALY, quality-adjusted life-year.

provision of healthcare. These costs could be associated with the diagnosis, treatment or management of disease (physician time, laboratory testing, hospital services, medication, etc.). Direct non-medical expenses are costs that are not inherently medical but still associated with the provision of healthcare, including costs of transportation, patient and caregiver time, training of technicians and medical professionals and hospital facilities and equipment. Intangible costs monetise other forms of burden, such as pain and suffering, and costs associated with lost productivity account for missed opportunities (in the workplace, in school, at home, etc.) attributable to disease. It should also be noted that different costs are often associated with different individuals within the healthcare system: the blood supplier, the hospital, the patient or the caregiver, for example.

In the blood collection example above, we focused on direct medical and non-medical expenses from the perspective of the blood supplier. If we had been thinking about costs from the donor perspective, or if we were using a broader societal approach, we would factor in indirect costs of lost productivity/wages and any pain or suffering associated with donation.

When thinking about the context of transfusion, it can be useful to organise costs into three categories: pretransfusion, transfusion and posttransfusion (Figure 50.1) [23]. Each of these stages consists of several distinct processes, all associated with costs, and the costs within each stage can be further classified into the categories described above: 'direct medical', 'direct non-medical' and 'intangible/productivity'. Furthermore, each cost can be associated with specific individuals or groups involved in transfusion.



	Pre-transfusion	Transfusion	Post-transfusion
Direct medical	• Donor Screening (S) • Donation Supplies (S) • Labour: Nurses, Physicians, Technicians (S, H) • Blood Processing (S, H) • Blood Testing (S, H)	• Patient Testing (H, P) • Transfusion Supplies (H, P) • Labour: Nurses, Physicians, Technicians (H, P)	• Patient Testing (H, P) • Post-Transfusion Supplies (H, P) • Labour: Nurses, Physicians, Technicians (H, P)
Direct Non-medical	• Donor Recruitment (S) • Donor Time (D) • Travel (S, D, H) • Storage/Inventory Management (S, H) • Facilities, Equipment (S, H)	• Patient, Caregiver Time (P) • Facilities, Equipment (H, P) • Travel (P)	• Patient, Caregiver Time (P) • Facilities, Equipment (H, P) • Travel (P)
Intangible or productivity-related	• Incapacity for work (D) • Pain/Suffering (D)	• Incapacity for work (P) • Pain/Suffering (P)	• Incapacity for work (P) • Pain/Suffering (P)

(S) = Blood Supplier (H) = Hospital (D) = Donor (P) = Patient

Figure 50.1 Representative costs associated with transfusion. Costs associated with transfusion can be categorised by the order in which they are experienced: pretransfusion, transfusion and posttransfusion. Within each of these categories, direct medical, direct non-medical and intangible costs are borne by donors (D), blood suppliers (S), hospitals (H) and patients (P). For hospitals that both collect and transfuse blood products, both the supplier and hospital costs would be incorporated. An economic evaluation may choose to focus on one of these perspectives and some or all of these cost categories. *Source*: Kacker et al. 2013 [23]. Reproduced with permission of John Wiley & Sons.

In addition, when conducting cost analyses (or any economic evaluation) over a period of years, outcomes may need to be discounted. Discounting incorporates the understanding that costs experienced in the future are worth less than those experienced today. This is because, given a choice, humans prefer to have an increase in money or health now, rather than later. While there is some debate over the value of discounting health effects (effectiveness, utility), it is generally accepted that future costs should generally be discounted at a rate of 3% per year [27]. Monetary outcomes also need to be expressed using a common base year (i.e. 2020 dollars), and this may require conversion of financial input parameters to a base year. Conversion between costs of different years often utilises the medical care component of the Consumer Price Index [28]. Depreciation of capital (equipment, facilities) may also need to be considered in some evaluations.

Cost-minimisation analyses are very similar to cost analyses, but compare multiple programmes or policies to select the least costly option. While the interpretation of these studies is generally straightforward, their exclusive focus on financial impact may not be appropriate or optimal for clinicians or policymakers also concerned with health impact. Building on our blood collection example, a blood supplier might be interested in holding a blood drive, but with minimal costs, and could use a cost-minimisation analysis to compare costs associated with alternative donation campaigns or venues.

Cost-effectiveness analyses account for financial impact as well as 'effectiveness', a natural measure of a health-related outcome of interest. Effectiveness is defined by the research team, and can vary from infections averted to adverse reactions prevented to lives saved to cases identified. Including a measure of effectiveness provides an additional dimension for the analyses, but because different analyses may use different measures of effectiveness, it is not always straightforward to compare alternatives. Continuing with our blood drive example, a blood supplier might want to minimise costs associated with their donation campaign, while also attracting the greatest number of donations. In this framework, they might be most interested in a cost per red cell unit collected, cost per donor, cost per new donor or some other measure incorporating effectiveness.

Cost–utility analyses resolve the issue of difficult comparisons by standardising the measurement of health outcomes in terms of quality-adjusted life-years (QALYs) or disability-adjusted life-years (DALYs). Health utility, in the form of QALYs or DALYs, is not a particularly concrete concept, but is a method used to compare various health outcomes using a single index [29]. The QALY is a measure of life-years discounted by a disability weight, such that one QALY is a year of life in perfect health. The DALY is a measure of the gap between life expectancy in perfect health and actual lifetimes, and is defined by $DALY = YLL + YLD$, where *YLL* is years of life lost due to premature death and *YLD* is years of life lived with disability. Cost–utility analysis is especially common in transfusion medicine literature related to interventions to reduce transfusion-transmitted infections or other potential transfusion risks. For example, transfusion services might be interested in methods of pathogen reduction to decrease transmission of HIV, hepatitis C virus (HCV), human T-lymphotropic virus (HTLV), bacteria, etc., and might use a measure of cost ($) per QALY to compare alternative interventions.

Finally, cost–benefit analyses go a step further by monetising the effects of a programme or policy, making the costs and benefits directly comparable in monetary terms. While this can be very helpful in decision making, the best method of monetising benefits may not be entirely clear.

Model Analysis

Since policymakers, payers, providers and patients frequently use economic evaluation to select between alternative investment decisions, these analyses are often set up as comparisons between proposed strategies (or technologies, interventions, programmes, policies, etc.) and baseline strategies, which could describe current methods, or another (generally less effective) option. In a simple decision analysis only two strategies are compared, but more complicated analyses may involve multiple options.

In the case that only two strategies are being compared (proposed versus baseline), net cost and net effectiveness are calculated for the proposed strategy, using the baseline strategy as comparison. For a given pair of compared strategies, four scenarios are possible, as illustrated in Figure 50.2 [26]:

1) The proposed strategy can be less costly and more effective than the baseline.
2) The proposed strategy can be more costly and more effective than the baseline.
3) The proposed strategy can be more costly and less effective than the baseline.
4) The proposed strategy can be less costly and less effective than the baseline.

In the first scenario, the proposed strategy is clearly preferable to the baseline strategy; it is optimal in terms of cost and effectiveness. In the third scenario, the baseline strategy is clearly preferable. However, in scenarios 2 and 4, the preferred strategy is less clear, since the proposed strategy is preferable in one dimension (health or financial) but not preferable in the other dimension.

When a decision involves multiple mutually exclusive proposals, the method of

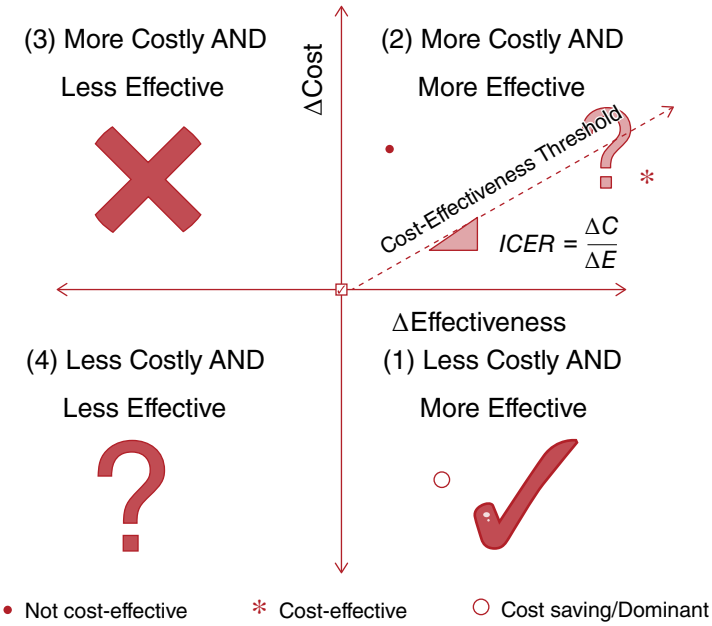


Figure 50.2 Illustration of cost-effectiveness outcomes. Each quadrant of this diagram represents one of four potential scenarios resulting from a cost-effectiveness analysis comparing one proposed strategy to a baseline strategy. The lower right quadrant (1) represents a scenario where the change in costs (ΔC) is negative (the cost of the proposal is less than the cost of the baseline), and the change in effectiveness (ΔE) is positive (the effectiveness of the proposal is greater than the effectiveness of the baseline). Any scenario falling in this quadrant will be cost saving and considered dominant over the baseline. The upper right quadrant (2) represents the situation where both ΔC and ΔE are positive. If the incremental cost-effectiveness ratio (ICER), calculated as shown, falls below a given threshold (e.g. $50 000/QALY [quality-adjusted life-years]), the proposed strategy is considered cost-effective. However, if the ICER is greater than the threshold, the proposed strategy would not be considered cost-effective. The upper left quadrant (3) represents the scenario where ΔC is positive and ΔE is negative. Any scenario falling in this quadrant would be excluded, since neither of the dimensions (cost or effectiveness) is enhanced under the proposed strategy. Finally, in the lower left quadrant (4), ΔC and ΔE are both negative. This is not a commonly considered scenario, since the proposed strategy being evaluated is generally either more costly or less effective than a baseline. *Source:* Kacker et al. 2013 [26]. Reproduced with permission of John Wiley & Sons.

analysis is similar. Typically, the proposed strategies could be arranged in order of increasing effectiveness, and an incremental cost-effectiveness ratio (ICER) is calculated. The ICER is defined as:

$$ICER = \frac{(C_1 - C_0)}{(E_1 - E_0)}$$

where C_1 and C_0 are the costs associated with a proposed strategy and a comparison strategy, respectively, and E_1 and E_0 are the effectiveness measures associated with each. The strategy used for comparison is generally the strategy with the next lowest effectiveness. No ICER would be calculated for the strategy with the lowest effectiveness. The ICERs associated with each strategy are then compared to determine if particular strategies can be eliminated, as demonstrated in Figure 50.3 [26].

While absolute thresholds for cost-effectiveness are often reported, there is not a single accepted value; benchmarks of $50 000, $100 000, $150 000 and $200 000 per QALY are generally accepted [29]. The role of these threshold values in transfusion medicine policy remains somewhat unclear;

Strategy	Costs ($)	Effectiveness (QALY)	ACER ($/QALY)	ICER ($/QALY)
Strategy 1 (Baseline)	0	0	---	---
Strategy 2	80	2	(80–0)/(2–0) = 40	(80–0)/(2–0) = 40
Strategy 3	160	4	(160–0)/(4–0) = 40	(160–80)/(4–2) = 40
Strategy 4	150	5	(150–0)/(5–0) = 30	(150–160)/(5–4) = –10

Strategy 3 is "strongly dominated": more costly and less effective than an alternative (Strategy 4)

Strategy	Costs ($)	Effectiveness (QALY)	ACER ($/QALY)	ICER ($/QALY)
Strategy 1 (Baseline)	0	0	---	---
Strategy 2	80	2	(80–0)/(2–0) = 40	(80–0)/(2–0) = 40
Strategy 3	160	4	(160–0)/(4–0) = 40	(160–80)/(4–2) = 40
Strategy 4	150	5	(150–0)/(5–0) = 30	(150–160)/(5–4) = –10

Strategy	Costs ($)	Effectiveness (QALY)	ACER ($/QALY)	ICER ($/QALY)
Strategy 1 (Baseline)	0	0	---	---
Strategy 2	80	2	(80–0)/(2–0) = 40	(80–0)/(2–0) = 40
Strategy 4	150	5	(150–0)/(5–0) = 30	(150–80)/(5–2) = 23.33

Strategy 2 is "weakly dominated": higher ICER than next most effective strategy (Strategy 4)

Strategy	Costs ($)	Effectiveness (QALY)	ACER ($/QALY)	ICER ($/QALY)
Strategy 1 (Baseline)	0	0	---	---
Strategy 2	80	2	(80–0)/(2–0) = 40	(80–0)/(2–0) = 40
Strategy 4	150	5	(150–0)/(5–0) = 30	(150–80)/(5–2) = 23.33

ACER = average cost–effectiveness ratio (Calculated using Strategy 1 as comparison group)

ICER = incremental cost–effectiveness ratio (Calculated using strategy with next lowest effectiveness as comparison group)

Figure 50.3 Illustration of example calculations and comparisons for cost-effectiveness ratios. In this example, the decision involves four mutually exclusive strategies, where Strategy 1 represents a baseline and Strategies 2–4 represent alternative proposals. Costs and effectiveness are shown for each of these strategies, and the proposed strategies are arranged in order of increasing effectiveness. The average cost-effectiveness ratio (ACER) for Strategies 2–4 is calculated using Strategy 1 as the comparison group, while the incremental cost-effectiveness ratio (ICER) is calculated using the next lowest effectiveness strategy as the comparison (Strategy 4 uses Strategy 3 as comparison, Strategy 3 uses Strategy 2 as comparison and Strategy 2 uses Strategy 1 as comparison). The ICER values are then compared to determine whether particular strategies can be eliminated. The top table shows that Strategy 3 is 'strongly dominated' by Strategy 4: Strategy 3 is less effective and more costly than Strategy 4. Thus, Strategy 4 is clearly preferable to Strategy 3, and we eliminate Strategy 3 from our list of potential strategies (second table). The ICERs are then recalculated, using Strategy 2 as the comparison group (next lower effectiveness) for Strategy 4. These results show that Strategy 2 is 'weakly dominated' by Strategy 4: the ICER for Strategy 4 is less than the ICER for Strategy 2 (third table). This indicates that the marginal cost of obtaining the effectiveness associated with Strategy 2 is greater than the marginal cost of an alternative. Strategy 2 is thus eliminated (fourth table). QALY, quality-adjusted life-years. *Source*: Kacker et al. 2013 [26]. Reproduced with permission of John Wiley & Sons.

expanded donor blood screening for HIV-1 using p24 antigen testing or nucleic acid testing has been shown not to be cost-effective in the context of most other medical interventions [30], but is still widely implemented. One study suggested that in the USA, nucleic acid testing for HIV, HCV and hepatitis B virus (HBV) in whole-blood donations is expected to cost between $4.7 million (£3 million) and $11.2 million (£7.2 million) per QALY saved [31].

Economic evaluations can also be incorporated into more complex models. Markov models, for example, are frequently used in scenarios where a process is recurrent or involves multiple stages or phases, such as

chronic transfusion therapy or screening for infection at fixed intervals. Markov models are state transition models defined by a set of mutually exclusive states – each can be associated with certain costs and effectiveness – and a set of transition probabilities between those states. Further description of these models is provided elsewhere [27].

Unrealistic assumptions in an economic evaluation can frequently lead to unreliable results, making it particularly important to keep in mind general guidelines for economic evaluations and to clearly report assumptions and methods, in addition to uncertainty. Nearly every parameter incorporated in a model – costs, probabilities, effectiveness measures – comes with some uncertainty or may vary over the time period analysed, and this uncertainty may affect the robustness of outcomes. Thus, any economic evaluation should address the impact of uncertainty in parameter estimates on reported outcomes. One-way sensitivity analysis is a method of varying only one input parameter (holding all other parameters fixed at 'base-case values') and reporting the resulting range in the outcome variable. A tornado diagram can be generated from a series of one-way sensitivity analyses, each varying a different parameter [26]. Two-way sensitivity analysis is also possible to modify two variables simultaneously. The extent of variation in the input parameters should reflect a range of generally realistic values.

Probabilistic sensitivity analysis is an additional method to vary multiple input parameters at one time. Each input parameter to be varied is described by a distribution (uniform, triangular, beta, normal, etc.), depending on its characteristics, and a value for each parameter is randomly selected from the appropriate distributions. The distribution underlying any input parameter is often unknown, and must be assumed by the research team based on expectations about the relative likelihood of different values for any particular variable.

Conclusion

Health economics, and especially economic evaluation, is becoming increasingly relevant in transfusion medicine [32]. It has previously been suggested that transfusion is somehow different from other areas of healthcare in that, as a society, we are willing to spend more for a given health improvement related to blood safety than we are willing to spend for a similar health improvement in other areas [33]. However, the costs associated with transfusion can be substantial, and demand persists despite implementation of patient blood management measures. In a context of increasingly restrictive budgets, applying the tools of health economics to transfusion medicine can and will be increasingly valuable.

KEY POINTS

1) Healthcare costs worldwide are rising, and the field of transfusion medicine is not isolated from these changes.
2) Health economics is concerned with effectiveness, efficiency and behaviour as they relate to the allocation of health and healthcare, and uses rigorous analytical methods to understand the behaviour of the many players within the healthcare system – patients, providers, public and private payers, communities, etc.
3) The field of transfusion medicine is increasingly relying on economic evaluation to help clinicians and policymakers maximise efficiency and patient benefit while minimising cost.
4) Multiple forms of economic evaluation exist, including cost analyses, cost-minimisation analyses, cost-effectiveness analyses, cost–utility analyses and cost–benefit analyses.

5) In the context of transfusion, it can be useful to organise costs into three categories (pretransfusion, transfusion and posttransfusion) and important to pay careful attention to which individuals or groups are affected by each cost.
6) Policymakers, payers, providers and patients may use economic evaluation to decide between alternative investments, and may incorporate this evaluation into different models to structure these comparisons.
7) Health economics, and especially economic evaluation, is becoming increasingly relevant in transfusion medicine. Applying the tools of health economics to transfusion medicine can and will be increasingly valuable.

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51 Scanning the Future of Transfusion Medicine

Jay E. Menitove[1], Paul M. Ness[2], Suzanne R. Thibodeaux[3] and Edward L. Snyder[4]

[1] *Community Blood Center of Greater Kansas City, Kansas City, MO (retired); Pathology and Laboratory Medicine, Kansas University Medical Center, Kansas City, KS, USA*
[2] *Transfusion Medicine Division, Pathology and Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD, USA*
[3] *Division of Laboratory and Genomic Medicine, Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA*
[4] *Department of Laboratory Medicine, Transfusion Medicine Service, Yale University, School of Medicine/Yale–New Haven Hospital, New Haven, CT, USA*

Transfusion medicine is a technology-based discipline undergoing continuous change. This chapter summarises recent significant changes and likely future changes to blood collection and component processing, hospital-based transfusion medicine and cellular therapies.

The field of transfusion medicine underwent a paradigm shift during the acquired immune deficiency syndrome (AIDS) epidemic. A similar paradigm shift is occurring as a result of the Covid-19 pandemic. Changes will need to occur in blood centres, as a substantial percentage of blood drives have been lost due to the closure of many businesses that used to host them. Blood centres will need to seek new ways to attract and recruit donors, many of whom do not wish to travel.

Changes in molecular diagnostics and therapeutics have occurred and doubtless eventually will affect transfusion medicine as well. New components and derivatives are being developed as technology advances. Automation, standardisation and a focus on quality and safety will continue to characterise blood component production. Pathogen-reduction technology will be growing as identification and pathogen-mitigation strategies – bacterial and viral – take centre stage. Blood product safety initiatives will require a perspective grounded in cost-effectiveness and informed by risk-based decision making. Patient blood management initiatives have reduced blood utilisation in developed countries, but the costs of improved components with reduced patient risk may be difficult to sustain and place community blood centres and national health service programmes in jeopardy. Increased data on clinical transfusion decisions will allow haemovigilance to improve patient outcomes.

Haematopoietic stem cell transplantation continues to evolve with respect to potential clinical indications. Minimisation of the toxicities of graft-versus-host disease remains a major goal. Other cellular therapies with the potential to treat cancer and non-malignant disorders, including those involving genetic modification, are rapidly

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expanding. Communication and collaboration of all parties involved in delivery of cellular therapies, including cellular therapy laboratories and transfusion medicine services, are of the utmost importance to optimise the chances for successful clinical translation of cellular therapies, now and in the future.

Blood Centre Perspective

This chapter in the previous edition identified four themes: (1) new technologies, (2) an adequate donation base, (3) decision making in uncertain and rapidly changing times and (4) fiscal sustainability of the blood supply. These topics retain relevancy and remain under discussion. However, the COVID-19 pandemic has altered practices and uncovered gaps that dramatically affect blood donation, data requirements, donor and product safety and innovation.

In the early spring of 2020, the COVID-19 public health emergency restricted commerce and travel. Elective surgery cancellations resulted in short-term inventory oversupply, which reverted to shortages once elective procedures resumed. Scheduled blood donation cancellations and donation venue reconfigurations to accommodate social distancing ensued. Donor- and staff-utilised hand sanitiser and personal protective equipment became available only through newly established vendor relationships. The quest for therapeutic interventions through passive immunisation resulted in COVID convalescent plasma (CCP) collections that required relationships with new donors, i.e. those recovering from COVID-19. Additional CCP regulatory, reimbursement and distribution issues followed. Taken together, pandemic-related changes provide the foundation for an emerging paradigm [1,2].

Business Model Adjustments

In response to the patient blood management–related downward trend in blood utilisation that began in 2008–09, blood collection agencies reduced donor outreach and investment in donor recruitment activities. During the past decade, blood donors' age trended towards a bimodal distribution. Younger and older donors represented the largest share; donors 25–60 years provided fewer donations proportionately. Overall, the median age of blood donors increased. Postulated reasons for the mid-age range donation deficit include work-related and childcare time constraints and lack of interest in blood donation. The latter implies a lack of donor engagement, especially by those in the post-high school age group. Future investments and research must focus on achieving an inclusive donor base by understanding donation-motivating factors and creating modern communication strategies targeted towards diverse demographic populations [3].

Expansion of existing or construction of new donor collection facilities located in proximity to donors' residences rather than businesses requires evaluation post pandemic if remote workplace activities or hybrid models persist. Additionally, blood collection agencies must increase their understanding of incentives encouraging first-time donations and those aimed at retaining donors. This includes the role, if any, of monetary payment for donations rather than recognition tokens or lottery and sporting game tickets [4]. SARS-CoV-2 antibody testing enticed some people to donate, although the demographics and intent to continue donations among these individuals require study.

In tandem, blood centres need more effective mechanisms for countering donation-associated fear, vasovagal reactions and other disincentives, in addition to current predonation educational materials, water loading and applied muscle tension techniques.

The current reimbursement model in the USA, in which blood centres receive payment for supplied products from hospitals that, in turn, obtain payment from insurance companies or governmental agencies, requires re-evaluation. National health

systems, present in most countries, provide funding for agencies producing blood components. The USA adopted this approach during the pandemic for emergency CCP production and distribution [1]. However, the routine fee-for-service approach in the USA appeared to be insufficient during the public health emergency. The extent to which the pandemic experience informs future reimbursement models that balance funding for innovation and improved patient outcomes with production costs currently remains uncertain.

Real-Time Data

Donor engagement initiatives require expansion of current techniques for achieving a balance between blood donations and hospital inventories. The pandemic demonstrated the added benefits of real-time data monitoring. Experienced donors responded to messaging about social distancing and proper hygiene practices implemented in response to COVID-19 concerns [2]. Coordinating hospital blood component utilisation with blood centre production forecasting, in real time, leads to improved inventory control, decreased wastage and greater efficiency [5].

Further alignment of patient outcomes with component recovery- and survival-related processing factors, and specific donor characteristics such as gender, smoking history, body mass index, metabolomics factors, phenotypic and genomic matching and other factors under investigation, will provide insights for identifying 'better donors' [6].

Additionally, data-derived analyses promote supply-and-demand alignment among blood collection vendors/suppliers. During the past decade, suppliers consolidated, resulting in fewer vendors with reduced manufacturing sites and less redundancy, a situation that poses supply-chain threats related to environmental or public health emergencies. Real-time and big data provide information about the location and availability of supplies. These data can potentially facilitate the development of virtual stockpiles for blood and supplies that, when periodically rotated, can minimise outdating and wastage [1].

Donor and Patient Safety

Haemovigilance systems, notably Serious Hazards of Transfusion (SHOT) in the UK, contain data for tracking and trending adverse events and monitoring the effectiveness of corrective actions. Similarly, monitoring donor responses to practices aimed at reducing vasovagal reactions, consequences of iron loss and plateletpheresis-related lymphopenia inform decisions about donor safety and donor-retention interventions.

The Transfusion-Transmitted Infections Monitoring System (TTIMS), a public–private partnership, monitors transfusion-transmissible infections in more than 60% of the US blood supply. TTIMS provided data about the impact of donor eligibility changes for males who have sex with men from a permanent to a 12-month deferral in 2016. The data demonstrating no significant change in viral transmission risk led to further revisions in 2020 to a 3-month deferral [7]. In the UK, the Standing Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) recommends sex-related donor-selection criteria. Effective mid-2021, SaBTO's individualised risk assessment, gender-neutral questioning replaces sexual activity questions previously asked only to males. TTIMS and the Recipient Epidemiology and Donor Evaluation Study (REDS) in the USA, SaBTO-related investigators in the UK, the Scandinavian Donations and Transfusions database (SCANDAT) and others focus on transfusion safety. They provide opportunities for measuring the impact of changes in donor qualifications. In the future, a logical extension of these monitoring systems includes evaluation of donor recruitment and retention messaging modifications on patient safety, with specific investigation of donors taking human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP).

Another monitoring activity, intended for improving transfusion effectiveness, involves blood component processing (manufacturing) changes, e.g. techniques used in preparing red cell and platelet components. Modifying the interval and transportation temperature between blood donation and component preparation, component density separation pressures, anticoagulant and additive storage solutions and storage temperatures affect transfusion outcomes [6]. Adopting modern data analytic approaches with a national or international scope supports product improvement, regulator review of these products and prompt implementation to improve patient outcomes.

Innovation

Near-term product improvements focus on safety, availability and alignment of product with the specific clinical situation. Large-volume delayed sampling, implemented in the UK and Canada, reduces platelet transfusion-associated sepsis. Buffy coat platelets, used extensively in the UK and Canada but not in the USA, provide significant logistical and operational benefits that augment platelet inventories. Cold-stored platelets extend platelet shelf-life for up to 14 days and reduce bacterial sepsis complications. Pathogen-reduction technologies (PRT), currently gaining acceptance in the USA, further reduce the latter hazard. However, PRT processing requirements reduce platelet availability. Low-dose PRT platelets – i.e. preparations containing 2.5–3.0×10^{11} platelets rather than $> 3.0 \times 10^{11}$ – obviate the restriction and maintain clinical effectiveness, but adoption of this approach may prove challenging in the USA.

Low-titre O whole blood (LTOWB) provides a product for a targeted patient demographic, those with acute traumatic injuries, by providing a single component. Establishing regional transfusion registries that maintain historical alloantibody information mitigates delayed haemolytic transfusion events for those patients receiving transfusions at more than one hospital, with evanescent alloantibodies detected at the first hospital but no longer detected by pretransfusion compatibility testing at the second hospital.

Mid-term innovations involve expanded adoption of blood donor red cell-alloantigen genetic testing to match chronically transfused patients, especially those with sickle cell disease who are at increased risk of Rh-antigen incompatibility. Donor human leucocyte antigen (HLA) loci genetic testing, in a similar fashion, improves outcomes in patients with alloimmune platelet transfusion refractoriness. Preliminary results of investigations identifying 'better donors' create opportunities for early adopters to measure the effectiveness of donor selection on patient outcomes. Importantly, anticipated regulatory approval of PRT red cells or whole blood complements the quest for significant transfusion-transmitted infection mitigation.

Longer-term innovation improvements include extended investigations of oxygen-carrying compounds for acute situations or for stockpiling in reserve for severe public health emergency-related blood shortages. *in vitro* pharmacological blood cell production remains under active investigation.

Hospital Transfusion Service and Patient Care Perspectives

Immunohaematological Progress

Future therapies will take advantage of the explosion of development in molecular testing of blood groups on red cells and other cellular components that are now applied to pretransfusion testing. Although the serological tests used to identify blood donor antigens and recipient blood groups and antibodies will probably not disappear from hospital and donor centre settings, the capability of these standard testing systems will be enhanced by automated methodology that reduces human testing errors and enhances turnaround time in transfusion services.

Many blood centres and transfusion services are using molecular red cell antigen-detection methods to screen blood donor inventories and to resolve difficult patient problems, where recent transfusions, autoantibodies or complicated transfusion histories make these testing systems a valuable and necessary adjunct to routine methods [8]. With these methodologies, blood centres are performing routine red cell genotype analysis that permits more specific donor–patient matching and will help hospitals to find blood for difficult-to-match recipients. Hospitals can also use molecular methods to better identify variants of the Rho (D) phenotype, to determine which women will need Rh immune globulin, thus avoiding the unnecessary treatment of many women that occurs with current testing protocols. Similar systems may enhance platelet transfusion therapy as well.

Although prospective matching has not been shown to reduce alloimmunisation for red cells or platelets in the past, even for high-risk patient groups, future studies are likely to continue to explore the utility of prospective matching. If shown to have value for patients, it will then be determined whether the clinical advantages justify the costs of these developments. Many hospitals have moved to extended matching of red cells for chronically transfused patients with sickle cell disease, based upon guidelines from the American Society of Hematology recommending matching for Kell and the Rh antigens CcEe. Enhanced antigen screening capability for cellular antigen systems, such as HLA, may prove to be particularly important for cellular therapies that will continue to grow rapidly in the future. Since the genetic basis of alloimmunisation remains elusive, these initiatives for patients at greater risk for antibody production may expand until we can differentiate between patients at risk and patients unlikely to be alloimmunised.

It is encouraging that a number of investigators are applying immunological methods in animal systems to determine how the process of alloimmunisation occurs, and whether there are therapies that could be applied to prevent alloimmunisation or reverse clinically significant alloantibodies in affected patients. Prevention or reversal of alloimmunisation to HLA would enhance solid organ transplant programmes, where previously immunised recipients are currently denied transplant options or required to undergo dangerous and expensive treatments to permit an incompatible solid organ or haematopoietic cell transplant.

Complications of Transfusion

As we take pride in this collective record of accomplishment and recent track record in mitigating transfusion-transmitted infections, complacency is not an option; diligence in reducing and eliminating transfusion risks must remain a primary transfusion medicine objective. In the USA, donor screening has been implemented to reduce *Babesia* transmission in states where donor prevalence is high. A number of blood safety concerns will be reduced by the adoption of pathogen-reduction systems, now available for platelets and plasma and hopefully available for red cells in the future. In the USA, the use of pathogen reduction for platelets is increasing with the finalisation of Food and Drug Administration (FDA) guidance on bacterial reduction, although many hospitals will rely on large-volume delayed sampling by blood centres or secondary testing at the hospital as other options [9]. Even if licensure of pathogen-reduced red cells is achieved, adoption may be stymied by cost considerations if pathogen reduction is advantageous for disease transmission issues alone. If these systems can be shown to reduce or eliminate some donor loss through travel history exclusions or elimination of unnecessary tests, or demonstrate other advantages for patients such as reduction of alloimmunisation or prevention of graft-versus-host disease, the case for adoption by transfusion services will be enhanced and reimbursement strategies will become more cogent.

A number of clinical trials have shown that older blood does not appear to harm patients, including a large study from Canada of 30 000 patients [10]. On the other hand, it remains difficult to perform randomised studies of blood near outdate, and the studies of older red cells in animals demonstrating worse outcomes remain a concern. Ongoing research will determine whether the suggested culprits of nitric oxide, microparticles, non-transferrin-bound iron or other biological modifiers can be manipulated by storage systems to reduce adverse effects for patients. Commercial development of new anticoagulants, washing systems or anaerobic blood storage may be proven valuable and implemented.

Our increased understanding of immunohaematological principles has improved our capability to reduce adverse transfusion complications. Persistent transfusion problems, such as delayed haemolytic transfusion reactions, TRALI not caused by donor antibodies, and allergic transfusion reactions should be amenable to detection and prevention by better use of our evolving knowledge of immunohaematology. It may also be possible to gain a better understanding of the pathophysiological mechanism and adverse effects due to immunomodulation, such that we can reduce this transfusion complication for patients.

Clinical Transfusion Practice

One of the positive outcomes from blood safety initiatives has been our response to regulatory pressures to standardise blood collection and preparation processes. Although there are clear benefits in terms of blood safety from standardised procedures, we have become increasingly aware that modifications in the components we transfuse are required to meet the unique needs of different patient populations. These modifications have created an opportunity to test new transfusion medicine concepts and practices with evidence-based methodology. Neonatal and paediatric transfusions have required hospital transfusion services to modify their practices to administer effective therapies in reduced volumes to these patients. Fresher blood components may be required for subsets of these patients, and blood components with reduced potassium loads for massively transfused children will be needed, perhaps prepared with potassium-reducing filters or better systems of cell washing. The availability of recombinant coagulation factors has revolutionised the care of haemophilia, and recently developed formulations with a longer half-life should enhance care. In a similar manner, new factors such as recombinant factor VIIa will continue to be introduced for broader patient groups with acute haemorrhage, but concerns about efficacy, toxicity and costs for these agents make the availability of yet-to-be-released newer recombinant proteins subject to prospective analysis and discussion. Fibrinogen concentrate use is growing, replacing the inconveniences of cryoprecipitate as a source of fibrinogen for acute bleeding, but its costs and regulatory issues have limited its adoption.

Data from the military suggested that early resuscitation using large volumes of plasma can save lives, leading to the development of massive transfusion protocols in hospitals, with red cells, plasma and platelets being administered in a 1 : 1 : 1 ratio. These practices are now being extended to other patients with major haemorrhage who clearly require red cell support. There are also publications demonstrating that the early infusion of plasma in trauma patients undergoing transport reduces mortality. It may be time to go back to the future and reinstitute the use of whole blood for these indications. Most of us incorrectly learned that whole blood does not provide platelet support, but evolving data demonstrate that whole blood stored in the cold maintains adequate functional platelets for at least 10–14 days. Whole blood provided from donors with low anti-A titres or filtered to remove isohaemagglutinins may make a better universal component for acute bleeding in the future.

At the same time as frozen plasma use is increasing dramatically in trauma, we recognise that frozen plasma is our most inappropriately ordered blood component, commonly used to correct trivial elevations of coagulation tests or prevent bleeding in procedures where evolving evidence has shown no medical value from this risky transfusion intervention. Complicating these issues are the many problems with plasma administration: ABO antibodies that make products unavailable as a universal therapy; large volumes that put patients at risk when acute care is needed; slow processing times due to thawing requirements; and inadequate potency for acute haemorrhage or reversal of anticoagulation. Plasma formulations that are concentrated, pathogen reduced and with low isohaemagglutinin titres should enhance therapy for acutely bleeding patients or those who are volume overloaded due to liver or cardiac disease. Lyophilised plasma products under development would increase the utility of plasma therapy.

Another plasma issue that is now recognised but perhaps inadequately addressed is the role of plasma therapy to correct the endotheliopathy of trauma [11]. In addition to its providing coagulation support, it is now recognised that plasma has a corrective role for this important clinical entity and that the corrective factor may not be maintained in some plasma formulations such as thawed plasma. Studies are underway to determine what plasma factor plays a therapeutic role and how best to deliver it via transfusion therapy.

The COVID-19 pandemic has led to the wide use of convalescent plasma collected from blood donors who have recovered from acute infection. When high-titre plasma is administered to patients early in the course of infection, the data that are evolving suggest efficacy. Use of this product in hospitalised patients may not be as efficacious. These plasma donations may also be used to prepare hyperimmune globulins, or perhaps be supplanted by monoclonal antibody preparations that are being developed.

Platelet therapy has been enhanced by recent developments such that platelets are widely available, alloimmunisation has been reduced by leuco reduction and managed by platelet matching, bacterial sepsis and other reactions have been reduced and platelet triggers and dosage are evidence based, at least for patients with haematological malignancies. Unfortunately, the advantages of platelet therapy have not been maximised for patients bleeding from trauma or surgery. Recent evidence suggests that the decision to provide all platelets with room-temperature storage may underserve bleeding patients, since 4 °C storage of platelets enhances haemostasis more promptly [12]. A large clinical study of cold platelets in cardiac surgery soon to be initiated should provide important guidance. Cold-stored platelets may also enhance transfusion support for acute bleeding in remote locations and may enable us to store platelets well beyond the current five-day period. Platelet particles or frozen platelets are also in clinical trials and may provide better haemostatic support.

Transfusion Medicine Consultation

These evolving medical transfusion issues suggest that the transfusion service will increase in importance as a source of product modifications, becoming more of a wet pharmacy for blood components. As a parallel development, transfusion services and their leadership will need to emphasise their critical role as transfusion consultants for clinicians, who will be faced with a growing menu of product modifications and new offerings from donor blood or the recombinant engineers. Many transfusion medicine specialists are now actively involved in the patient blood management movement; in addition to its focus on reducing unnecessary transfusions, the clinical interactions that will result should provide better opportunities for transfusion specialists to help clinicians target the unique needs of their patients. As we embrace the growing heterogeneity of the products we offer from donor blood, recombinant

proteins, cellular engineering and bone and tissue banking, and continue to offer these services with emphasis upon our critical consultative role, the transfusion medicine discipline will continue to grow and flourish, with benefits to patients and their supporting clinicians. To sustain the recognition of these important functions of transfusion specialists, continued involvement of our practitioners in therapeutic apheresis and cellular therapy should be emphasised to reinforce the value of our involvement to clinicians in need of our services.

Alternatives to Transfusion

The transfusion community will continue to learn from patients who refuse blood therapy, and treatment modifications that provide care to these patients that results in outcomes that are similar to if not better than patients accepting blood transfusions will continue to be recognised. Bloodless medicine programmes have emphasised the development of impeccable surgical technique, the recruitment of physicians willing to care for these patients with an understanding of this therapeutic limitation, the use of transfusion alternatives, the restriction of transfusions to lower triggers based upon evolving clinical evidence and the need for presurgical assessments and informed consent discussions with patients well in advance of surgical procedures.

Our enthusiasm for transfusion alternatives will hopefully be rewarded with new therapies in the future. Tranexamic acid use will continue to grow and fill the gap created by the withdrawal of aprotinin from clinical use. The long search for a haemoglobin-based oxygen carrier (HBOC) to replace blood in trauma was impaired by clinical evidence from a major trial in trauma showing limited efficacy and a meta-analysis demonstrating that the HBOC class has adverse effects of increased myocardial infarctions and mortality compared to controls as a result of nitric oxide effects. The continuing clinical need for patients with severe anaemia who cannot be transfused due to autoantibodies, alloantibodies, hyperhaemolysis or religious objection will hopefully be fulfilled as increased knowledge of haemoglobin function and nitric oxide effects is applied to modifications of old products or development of new formulations, including engineered red cells.

The Future of Cellular Therapy

The origins of cellular therapy in transfusion medicine began in the nineteenth century with transfusion of whole blood, and blood and blood component transfusion has since become a mainstay of life-saving and life-sustaining therapy. The field of cellular therapies, including potentially curative therapies such as haematopoietic stem cells (HSCs) collected from different sources and genetically modified T cells, is intricately related to the specialty of transfusion medicine, which typically includes apheresis, blood banks, cellular therapy laboratories or a combination thereof. Cellular therapy products may be collected by apheresis, subsequently processed or undergo manufacturing by cellular therapy laboratories, depending on that laboratory's capabilities, and stored for infusion if needed. In addition to the expansion in types of cellular therapies offered as treatment options, the role of transfusion medicine services continues to develop as a critical component of cellular therapy care delivery.

Cellular Therapies That Undergo Minimal Manipulation

HSC transplantation has been steadily improving in many ways. The number of clinical indications continues to expand and include malignancies of haematological and non-haematological aetiologies as well as non-malignant conditions of genetic origin, such as inborn errors of metabolism, and acquired aetiologies, such as autoimmune diseases, including multiple sclerosis [13].

Patients considered as potential HSC transplant candidates are of an increasingly large age range, and their survival and outcomes are continually improving. Many countries have HSC transplant registries allowing for international donations, expanding the geographical reach and donor pool for potential HSC transplant recipients [14].

In addition to improvements in conditioning regimens and supportive therapies, efforts to improve cellular therapy product procurement and processing continue to contribute to the mitigation of short- and long-term adverse events. Most donors provide HSCs from peripheral blood, followed by bone marrow harvest and cord blood [15]. The least-utilised source of HSCs, cord blood, can be stored long term. Cord blood transplantation requires less stringent HLA matching and inventory availability, and is ongoing via cord blood donation at the time of delivery by non-invasive means. Therefore, continued investigation regarding the potential applications of cord blood could benefit the field, especially in times where stem cell donation is disrupted on a large scale, as demonstrated by the COVID-19 pandemic. The relatively high number of lymphocytes in HSC products from peripheral blood as well as the popularity of its use highlight the continued importance of cellular processing and therapy development to prevent or reduce the incidence and/or severity of graft-versus-host-disease.

Improvement of current processes, development of alternative cryopreservation solutions, CD34+ cell enrichment or depletion of selective T-cell subsets are examples of forward progress in cellular therapies in the realm of standard of care. Continued investigations into potential applications, such as HSC transplant for sickle cell disease or autoimmune diseases, could transform the goals of care for some clinical conditions from therapeutic to potentially curative. Taken together and with the continual progress of medicine as a whole, HSC transplant continues to be a mainstay in cellular therapies and clinical applications will likely continue to expand as morbidity and mortality associated with HSC transplantation continue to improve.

Cellular Therapies That Undergo More Than Minimal Manipulation

Novel cellular therapies are continually being investigated for potential clinical application and undergo extensive evaluation before being deemed suitable for clinical use; many of them do not ultimately obtain clinical approval. As examples of regulatory models overseeing product development, cellular therapy products classified as 'more than minimally manipulated', as defined by the FDA [16], or as advanced medicinal therapeutic products by the European Medicines Agency [17], undergo thorough vetting through each respective group's clinical approval process.

Chimeric antigen receptor (CAR)-T cells represent a significant breakthrough as the first genetically modified cellular therapy product to obtain approval for clinical use [18]. CAR-T cells are autologous, genetically modified T cells engineered to express a chimeric antigen receptor, a protein containing a single variable chain fragment directed at a particular target coupled to downstream T-cell signalling. This effectively pairs the antigen specificity of B cells with the cytotoxic function of T cells. Clinically approved CAR-T cells have been directed against CD19, with clinical indications including CD19-expressing haematologic malignancies, and their clinical use is growing. Clinical development of CAR-T cell products directed against other targets has since been extended. A CAR-T cell directed against B-cell maturation antigen (BCMA), the first target other than CD19, was recently approved for multiple myeloma, demonstrating the realisation of an expanded application of CAR-T cells to other diseases [19]. Refinement of the technologies and manufacturing processes, including gene delivery such as with CRISPR/Cas9, and the development of allogeneic CAR-T cells, identification of additional new

therapeutic targets and continued improvement of those already in clinical use will all continue to be important clinical developments in CAR-T cell delivery. Improved patient care, including management of the adverse effects associated with CAR-T cell therapies such as tocilizumab for the treatment of cytokine release syndrome and long-term follow-up of patients who have already received CAR-T cell therapies, will inform the cellular therapy community and guide future directions for this preeminent cellular therapy product [20].

Other cellular therapy products that undergo *ex vivo* manipulation have the potential for future clinical application. T-cell products such as viral-specific T cells could help mitigate the risk of severe viral infections during the HSC transplant process. Tumour-infiltrating lymphocytes and other immune cells such as natural killer cells could provide additional cancer therapeutic options. HSCs that have been modified to alter disease-causing genetic mutations in blood cells are under development and have the potential to have a profound impact on haemoglobinopathies, such as offering a potential curative option for sickle cell disease [21]. These are just a few examples of potentially paradigm-shifting candidates for clinical application that hold great promise for the future of cellular therapies.

The Evolving Role of Transfusion Medicine in Cellular Therapies

Transfusion services can vary with respect to the level of direct involvement, but nonetheless play a vital role in cellular therapies. Depending on the clinical environment, services provided by transfusion medicine may range from the collection of cellular therapy products by apheresis to cellular therapy laboratories that minimally manipulate products, or those that manufacture more than minimally manipulated products. The entire process requires coordination of care and collaboration among many different teams involved in patient care.

There are some practical considerations for transfusion services that can help ensure perpetual readiness for the growth of clinical cellular therapies. Estimating current workload and anticipating future capacity form an essential and foundational component of the successful and safe everyday operation of cellular therapy product collection and processing. Anticipating changes in workload, roles and responsibilities in response to more products of different types and processing requirements is critical, so that processes and people can adapt accordingly. The development of educational pathways for future cellular therapy technologists, those with regulatory and research oversight, as well as laboratory directors and medical directors will also allow for continued sustainable progress of the field.

Consideration of space, materials and equipment is essential for the ever-expanding volumes of cellular therapies. Standard-of-care HSC transplant products can be cryopreserved, usually for a period of multiple years. Given the increasing survivability of HSC transplantation, cellular therapy labs that store HSC products must look ahead to ensure that the storage capacity can continue to accommodate the compounding addition of products to their inventory, or find ways to adapt to do so. Expansion of space, potential renovation or new construction to allow for safe spaces to manipulate products may also be required as cellular therapies become increasingly complex with respect to processing needs.

Coordination is critical to the successful delivery of care involving cellular therapies, and this will only continue to gain importance as the field expands. Timing of patient preparation, product collection, product processing and manufacturing and product infusion are all factors commonplace for cellular therapy products, regardless of clinical approval status. Support of cellular therapy products that have yet to obtain clinical approval is becoming an ever-present factor in clinical operations, highlighting the progress that cellular therapy research has made

and the potential for clinical translation. Cellular therapies were developed as unique protocols, but often the protocols were adapted by extension to multiple products. Even if processes are relatively similar, working on multiple cellular therapy products that differ even slightly in processing requirements can have profound effects on a given workflow and operation. The involvement of transfusion services as early as possible when clinical studies are being considered can be immensely helpful for planning purposes for any future products being developed for clinical application. On a higher level, the development of clinical guidelines for future cellular therapy product development as it relates to the transfusion medicine service perspective could translate to faster, smoother realisation of protocols into processes, and by extension delivery of these therapies to patients.

As new cellular therapies are introduced as therapeutic options and as the volume of products already established for clinical use continues to expand, the cellular therapy laboratory must continue to grow in parallel and in coordination with clinical services with respect to the expanding cell therapy repertoire. Accounting for the expanding nature of cellular therapy products considered standard of care, as well as the exponential growth of those in clinical development, is critical for future success. The development of proactive approaches, including guidelines and regulations, to the ongoing assessment of how cellular therapies fit into transfusion services and cellular therapy laboratories will help foster the successful delivery of cellular therapies. The future of transfusion medicine as it relates to cellular therapies is bright and the possibilities are seemingly endless. Forward thinking and collaboration can help ensure the successful translation of any potential future cellular therapy product in this exciting time of cellular therapy medical innovation. Key points to consider are the following:

- Standard-of-care cellular therapy products are expanding in clinical indication and potential candidates, resulting in growing volumes seen by transfusion services.
- Cellular therapy process development, adaptation and anticipation of growth are critical to fostering the continued growth and improvement of cellular therapy care delivery.
- Communication and multidirectional collaboration among all involved parties, including transfusion medicine services, is vital to the ongoing evolution of cellular therapy product development and its successful clinical translation.

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