Melanie Coathup   *Editor*

Musculoskeletal Infection

Springer

Melanie Coathup

Editor

Musculoskeletal Infection

Springer

Editor
Melanie Coathup
College of Medicine
University of Central Florida
Orlando, Florida, USA

Contents

About the Editor

Melanie Jean Coathup is Professor of Medicine at the College of Medicine in the University of Central Florida and Director of the Biionix (Bionic Implants, Materials and Interfaces) Cluster. Dr. Coathup qualified in Medical Cell Biology from the University of Liverpool, United Kingdom and gained a PhD in orthopaedic implant fixation at the Institute of Orthopaedics, University College London (UCL) based at the Royal National Orthopaedic Hospital in Stanmore, UK. She became Head of the Center for Tissue and Cell Research at UCL and Divisional Head of Athena SWAN and Women in Science. Over the years, Dr. Coathup has worked in Biomedical Engineering and orthopaedic innovation with the view of investigating and applying scientific discovery to improve the treatment and care of patients. During her career, her research has focused on improving orthopaedic implant fixation and enhancing bone regeneration, focusing on translational themes that include stem cell therapy, nanotechnology, the design and follow-up of implants, implant infection and novel osteostimulative materials and coatings.

Chapter 1
The Musculoskeletal Burden: Where Are We Now?

Abinaya Sindu Pugazhendhi, Fei Wei, and Melanie Coathup

Abstract The dramatic increase in average life expectancy during the twentieth century ranks as one of the society's greatest achievements, and the world's older population continues to grow at an unprecedented rate. As the proportion of older people, length of life, and health expectations continue to increase, a rise in nonfatal age-related musculoskeletal degenerating diseases, disability, and prolonged dependency is projected. To manage this rise and in order to restore or improve pain-free activity, independence, and quality of life, a significant increase in the number of musculoskeletal surgical and nonsurgical encounters is forecast. As such, the incidence of musculoskeletal infections will also increase, where many will be challenging, complicated, and costly to treat. This chapter describes the impact and growing burden of musculoskeletal disorders, summarizes our current societal challenges, and overviews trends in the ever-growing orthopaedic device market. The chapter also highlights recent successes that have improved our understanding of how to treat musculoskeletal infections as well as the many multifactorial challenges that remain.

Keywords Aging population · Musculoskeletal · Orthopaedic · Burden · Device · Infection · Disorders

1.1 The Global Rise in Our Aging Population

The dramatic increase in average life expectancy during the twentieth century ranks as one of the society's greatest achievements. Although most babies born in 1900 did not live past age 50, life expectancy at birth now exceeds 83 years in Japan the current leader, and is at least 81 years in several other countries [1]. As a result, the world's older population continues to grow at an unprecedented rate. Today, 8.5% of people worldwide (617 million) are aged 65 and over, and according to a new

A. S. Pugazhendhi · F. Wei · M. Coathup (✉)
Biionix Cluster & College of Medicine, University of Central Florida, Orlando, FL, USA
e-mail: melanie.coathup@ucf.edu

report, this percentage is projected to rise to nearly 17% by 2050 (1.6 billion) [2]. As the global number of centenarians is expected to increase tenfold between 2010 and 2050, we soon will have a greater number of older people than children under the age of 5 and more people at extreme old age than ever before [1]. Musculoskeletal health is critical for human function, enabling mobility, dexterity, and the ability to work and actively participate in all aspects of life. However, as the proportion of older people, length of life, and health expectations continue to increase, a rise in nonfatal age-related degenerating diseases, disability, and prolonged dependency is projected, in particular, in musculoskeletal disorders (MSDs), as they cause the second highest volume of functional impairment and highest, in terms of years lived with disability (YLD) in the adult population globally and more so than any other disorder group [3]. This is because musculoskeletal conditions are prevalent among all age and gender groups, across all sociodemographic strata of society. MSDs include more than 150 diagnoses that affect the locomotor system and encompass diverse conditions that affect our muscles, bone, connective tissue, and joints through inflammatory or degenerative processes and infectious, traumatic, or developmental events or as a result of toxic/metabolic diseases, neoplasms, or vascular diseases. These conditions are characterized by pain and reduced physical function, which affects individuals by limiting their activities and restricting their participation, while also affecting societies through work loss, disability pensions, early retirement, and the increasing need for social support. Chronic MSDs can also aggravate other disease conditions due to their activity-limiting effects, including respiratory and cardiovascular disease [4, 5]. Presently, ~1.71 billion people live with an existing musculoskeletal condition globally, and given our aging population and longer life expectancy, it is anticipated that nonfatal musculoskeletal diseases will pose a major future societal and healthcare concern [3, 6].

To manage this projected rise in MSDs, and in order to restore or improve pain-free activity, independence, and quality of life, a significant increase in the number of orthopaedic surgical encounters is forecast. The long-term success of orthopaedic treatment will aid in allowing people to live a healthy, active, and independent lifestyle well into old age. This may mean that patients with soft tissue rheumatism, arthritis, a joint prosthesis, and other age-related comorbidities may seek to work beyond the traditional retirement age as well as provide individuals the opportunity to pursue new activities such as further education, enable a new career, or pursue a long-neglected passion.

1.2   The Global Burden of Musculoskeletal Disorders

The burden of MSDs has been estimated to span five major diseases: rheumatoid arthritis (RA), osteoarthritis (OA), lower back pain, neck pain, and gout. Other MSDs include osteoporosis and fragility fracture, infectious arthropathies, inflammatory polyarthropathies, disorders of the tendon, and regional pain syndromes such as those following an injury or activity associated with sports or occupation.

As mentioned, the prevalence of MSDs is substantial and increasing worldwide. Globally, the incidence of the five major MSDs increased from 211.8 million to 334.7 million (58%) between 1990 and 2017 [7, 8]. Additionally, there were ~ 336.5 million cases of "other" MSDs reported globally in 2017, with a higher prevalence in females, and with ~74,000 associated deaths and ~ 30.8 million disability-adjusted life years (DALYs), an increase of 7.2% and 3.4%, respectively, between 1990 and 2017 [9]. According to the Global Burden of Disease data, DALYs due to musculoskeletal conditions increased by 61.6% between 1990 and 2016. Specifically, osteoarthritis was associated with a 104.9% rise in DALYs [10], and lower back pain remains the leading cause of global disability since 1990.

In the United States, the National Health Interview Survey (NHIS) estimated that between the years 2013 and 2015, 126.6 million, i.e., 1 in 2 adults, were affected by a MSD. This is twofold higher than the population affected by pulmonary or heart disease [11]. Annual costs of 5.76% GDP (~$980 billion) covered treatment costs and lost wages, with direct costs of $53.1 billion attributed to treat injuries that culminated in ~264 million lost workdays and an annual earnings loss of $131.8 billion [11]. Approximately 19% of the US population visited healthcare providers, which equates to ~235.1 million visits, due to low back pain (61.8 million), hospitalizations for arthritis and other RA conditions (6.4 million), injuries (62.7 million), and childhood injuries (10.6 million). Similarly, MSDs accounted for 21% of all incapacity benefit claims filed in the United Kingdom and over 30% of new long-term sick leaves and disability pensions in Finland and Sweden [4].

As such, MSDs are the predominant, most costly and disabling condition in the United States [12]. Osteoarthritis and RA are known to significantly impact quality of life, including general health, physical health, and mental health, as a result of lack of independence, reduced physical activity, pain, sleep, and loss of well-being [7]. However, recent studies have shown that OA is becoming the most common cause of disability for middle-aged Americans and has become the most common cause of disability for people older than 65 years, affecting more women than men [12, 13]. Joint replacement surgery is performed to restore function and relieve pain in patients with severe OA, and contemporary statistics show that 60% of all MSD-related surgical procedures are associated with joint reconstruction surgery. Presently, there are approximately 55.4 million adults estimated to be living with arthritis in the United States [14], and based on NHIS data, it has been estimated that by 2040, 1 in 4 adults (~78 million) will be diagnosed with arthritis. It is anticipated that 44% will report activity limitations attributed to their arthritic condition with an annual earnings loss of ~$71.3 billion [15].

Although most common in postmenopausal women and due to hormonal decline, a degree of age-related osteoporosis is inevitable in both men and women. In 2020, approximately 12.3 million individuals >50 years of age in the United States live with osteoporosis, and the incidence of osteoporotic hip fractures alone is predicted to increase from 1.66 million to 6.26 million by 2050 [16, 17]. In the United States, an estimated 2 million osteoporosis-related fractures occur each year prompting more than 0.5 million hospitalizations, 0.8 million emergency room encounters, and 2.6 million physician office visits, and are associated with a decreased quality of life

[16]. Additionally, the annual cost of treating a hip fracture in the late 1990s was US$34.8 billion, and this is projected to exceed US$130 billion by 2050 [18]. Of note, people living with RA and OA and those who have sustained an osteoporotic fracture have higher mortality rates than their age- and gender-matched peers [19].

1.3 Current Societal Challenges

Musculoskeletal tissue can be considered a complex physiological hub of tissue that responds to several stimuli of different origin (e.g., mechanical loading, lever action through traction of muscles, diet, the immune, endocrine, and nervous system). Their integration directly regulates the composition, microarchitecture, and volume of tissue, thereby its structural strength, as well as influences the release of hormones and various mediators that communicate with the rest of the body. The major determinants of musculoskeletal health are considered to be age, gender, obesity, work burden, physical inactivity, smoking, excess alcohol, and injury. Examples of some of these determinants and their importance are briefly highlighted in Sects. 1.3.1–1.3.4.

1.3.1 Physical Inactivity

Exercise affects all human tissues and organs, and physical activity is the key stimulus for bone and muscle metabolism. Bone and muscle cell activity is stimulated through both direct and indirect mechanical loads mediated through weight bearing and muscle traction, respectively, as well as by endocrine stimulation or via the immune system [20]. Despite the overall health benefits of regular physical activity, people aged 55 years and older are regularly identified as the most sedentary group in the population, worldwide. Most studies report that between 40% and 80% of older people do not meet the recommended guidelines [21, 22]. Inactivity rates increase with age, with around two-thirds of those aged between 65 and 74 years, and three-quarters of those over 75 years, not meeting the recommended guidelines of 150–300 min of moderate-intensity aerobic physical activity or 75–150 min of vigorous-intensity aerobic physical activity weekly (or an equivalent combination) [23, 24]. In addition to this, the current pandemic of sedentary behavior, in part due to the rapid rise in video gaming, social media, and video-streaming, has resulted in nearly 30–39% of the US population engaging in below minimum levels of recommended daily aerobic activities and exercise [25]. This rise in physical inactivity is considered a major risk factor for a number of chronic diseases, including type-II diabetes, obesity, and coronary artery disease, in many countries globally [26–28]. Further, inactivity has risen to above 50% of the population in some countries. Obesity is a major contributor to joint degeneration, and diabetes is an important predictor for severe forms of arthritis. Diabetes has also been shown to be an

independent risk factor for the progression of OA in men [29, 30]. Thus, increased physical activity will reduce the numbers in the population susceptible to obesity, MSD, ill health, and injury due to falls.

The idea of exercise for the rehabilitation of MSDs has been widely accepted for many years, by improving locomotor function, balance, and strength. For example, currently, the American Academy of Orthopaedic Surgeons guidelines recommend that patients who have symptomatic knee OA engage in quadriceps strengthening and low-impact aerobic exercise. The beneficial effect of exercise has been shown to reduce pain in patients with knee osteoarthritis [31], and stiffness in those suffering with back pain [32] as well as increasing bone mineral density and muscle strength and by reducing inflammatory markers in osteoporotic patients [33].

1.3.2 Obesity

Obesity is a chronic, multifactorial, polygenic health threat of increasing global concern [34, 35]. In adults, the World Health Organization (WHO) defines overweight as a body mass index (BMI) ≥25 and obesity, ≥30. A recently updated report from the WHO highlighted that worldwide, obesity nearly tripled between 1975 and 2016 [36]. This 2016 report estimated that globally, 39% of adults (1.9 billion) were overweight and 13% (>650 million) were obese. Among these, women were more prone to being overweight or obesity, than men. The report also estimated that 340 million children and adolescents aged 5–19 years, and 41 million children <5 years of age, were overweight or obese globally. A systematic analysis carried out in 2013 estimated that the proportion of adults with a BMI ≥25 increased from 28.8% in 1980 to 36.9% in 2013, in both developed and developing countries, with estimated annual direct costs of $100 billion in the United States alone [37]. This study also showed that the incidence of overweight and obese children also increased from 8.1% to 12.9% in boys and 8.4% to 13.4% in girls in 2013. Based on this current trend, a global epidemic is forecast, and it is estimated that up to 50% of the population will be classified as overweight or obese by 2030 [38].

In adults, the association between obesity and an impaired soft tissue healing response has been widely reported. Being overweight significantly increases the likelihood of infection-related complications due to decreased vascularization of the adipose tissue, where poor perfusion limits the delivery of nutrients as well as the host immune cells necessary to combat microbial species at the site of repair [39, 40]. Additionally, obesity and osteoporosis are intimately related. Initial studies described a benefit to skeletal health due to the increased weight imposed by adipose tissue providing supplementary mechanical load onto surrounding bone [41]. However, more recently, new insights view obesity as an important risk factor for osteoporotic fragility fractures at several anatomical sites [42].

Obesity is also associated with the development, progression, and symptomatic severity of osteoarthritis of the knee and is one of the most modifiable risk factors for OA [6]. Metabolic syndrome, characterized by an accumulation of metabolic

abnormalities, is often closely related to overweight, obesity, and inactivity [43]. This, in turn, may increase the risk of developing diabetes, OA, neurological complications, and atherosclerotic and nonatherosclerotic cardiovascular disease [44]. Additionally, previous studies have indicated that obesity causes persistent low-grade inflammation [45], where excessive adipose tissue and immune cell infiltration (e.g., macrophages, T cells) are considered primary initiators of inflammation when under obesity and metabolic disease conditions [46]. As a result, adipocytes and immune cells release a spectrum of pro-inflammatory adipokines and cytokines [47], most of which may contribute to the structural and biochemical changes in articular and musculoskeletal tissues [48]. Increased BMI is also a known risk factor for developing RA, with a 13% increase in risk reported for every 5 kg/m^2 increase in BMI [49].

1.3.3   Age and Gender

The incidence and rate of progression of MSD increases with age and peaks in the 65–69-year age group for both men and women [9]. Gender differences vary by age, for example, MSDs are more prevalent in men when under 45 years of age however, above age 45, more women than men are reported with MSDs [13]. Back pain and musculoskeletal problems related to injuries are more common in men, while women are at a greater risk of developing rheumatoid arthritis, osteoarthritis, and osteoporosis and sustaining a fragility fracture. In women, the incidence of OA is highest among those aged 65–74 years, reaching approximately 13.5 per 1000 population per year [9]. In the male, the incidence is reduced, and approximately 9 cases per 1000 population per year occur in those aged 75 years and over [50].

1.3.4   Diet and Nutrition

Diet is important in both the prevention and progression of musculoskeletal conditions. Dietary intake plays a primary role in bone and soft tissue metabolism and has a significant impact on tissue health, structural integrity, and repair. Eating a varied diet high in fresh fruit and vegetables is recommended by many health organizations. Vitamins D and K, calcium, and protein optimize muscle, bone, and functional outcomes in people, thereby reducing falls and fractures [51–54]. However, there was a notable transition in diet after the Industrial Revolution in the eighteenth century, with a change from a more polyunsaturated, high-fiber diet to a diet high in saturated lipids and trans-fatty acids, with decreased levels of vitamins C and E, an eating pattern termed the Western diet. Presently, the consumption of the unhealthy Western diet is increasing worldwide, and these highly processed convenience foods are high not only in saturated and trans-fats but also in sugars and salt. It has been widely established that a high saturated fat diet is associated with a number of

diseases due to the chronic low-grade activation of pro-inflammatory pathways and adipogenesis, where in bone tissue, for example, this leads to osteoclastogenesis and subsequent bone resorption. The health benefits of PUFAs and the role of ω-3 present in fish oil, in particular, have been shown to be critical in the healthy development of the infant nervous system and retina [55]. Evidence of the beneficial effect of ω-3 and ω-6 fatty acids has led to the establishment of comprehensive recommendations and the dietary reference intakes report from the Food and Nutrition Board in the United States. In terms of bone health, recent research suggests that polyunsaturated fats, particularly those high in ω-3 fatty acids, are able to interact with both hematopoietic and stromal derived bone cells and elicit significant anti-inflammatory properties including inhibition of osteoclastogenesis while promoting osteoblastogenesis thereby suppressing bone resorption and increasing bone regeneration, improved microarchitecture, and structural strength.

In a cross-sectional study involving 1209 adults aged 20–30 years, it was found that participants consuming sugary drinks rich in fructose such as high-fructose corn syrup, sweetened soft drinks, fruit drinks, and apple juice at least 5 times a week demonstrated a threefold increased risk of developing arthritis, irrespective of other dietary factors, plasma glucose levels, physical activity, or smoking [56]. Moreover, there are single dietary factors that have proven important in musculoskeletal health. A study by Pattison et al. [57] showed that daily consumption of a glass of freshly squeezed orange juice inversely correlated with the risk of RA, possibly due to the protective action of beta-cryptoxanthin, a natural carotenoid and antioxidant. There are clear associations of many micronutrients, including zinc, vitamin C, and vitamin A, with various aspects of wound healing and recovery from injury, including muscle disuse [58]. For example, zinc is important for human health and disease due to its critical roles in growth and development, bone metabolism, the central nervous system, and immune function. Additionally, zinc plays a major role in regulating every phase of wound healing, ranging from membrane repair, oxidative stress, coagulation, inflammation and immune defense, tissue reepithelialization, and angiogenesis to fibrosis/scar formation deficiency. A zinc deficiency is associated with delayed wound healing [59]. Similarly, copper deficiency is associated with osteochondrosis and subchondral bone changes [60]. Thus, nutritional support may be crucial to lessen the length of time and reduce the negative aspects of MSDs.

The 10^{14} microorganisms found within the gut microbiota appear to be a significant contributor to musculoskeletal health and are a growing area of research for health promotion, disease prevention, and disease treatment. The gut microbiome, composed of mainly bacteria, but also viruses and fungi, produces a large and diverse pool of bioactive small molecules that are able to establish a systems-level connection with the host metabolic, endocrine, immune, and nervous systems [61]. Therefore, this, in turn, influences the pathophysiology of several distant organs including the skeletal muscle and bone. Physical activity is a possible modulator of intestinal microbiome composition, and the microbiome has been shown to affect bone metabolism and subsequently bone quantity, quality, and strength. Preclinical work has also demonstrated an effect on the success of osteoporosis, osteoarthritis,

and muscle mass. For example, and using animal models, the absence or significant reduction of a gut microbiome has been shown to prevent bone loss due to estrogen depletion and glucocorticoid treatment, two primary contributors to the progression of osteoporosis [62–64]. This is further supported by a randomized, double-blinded placebo-controlled multicenter trial carried out in 2019 ($n = 249$ individuals), which showed that after 1 year, those individuals who received daily oral probiotics demonstrated reduced postmenopausal bone loss in the lumbar spine [65]. Alterations to the gut microbiome have also been shown to affect OA. Following surgically induced osteoarthritis through destabilization of the medial meniscus in a murine model, changes to the gut microbiome prevented cartilage loss in germ-free mice [66] as well as cartilage loss and limited obesity-induced joint degeneration [67]. Skeletal muscle mass and function has also been shown to be affected. A recent murine study found that the muscle atrophy displayed in germ-free mice who lacked a gut microbiota was reversed following the transplantation of a gut microbiota obtained from normal, pathogen-free mice [68]. This study reported that transplantation of the gut microbiota resulted in an increase in muscle mass, a reduction in muscle atrophy markers, and an improved oxidative metabolic capacity of the muscle.

Through the bodily distribution of microbial products and proteins, the gut microbiome regulates the host immune system, and is able to influence the hosts' resistance to infectious disease [69–71]. For example, in mice, the 99–100% depletion of the gut microbiota led to a severe reduction in phagocyte populations, making animals more susceptible to infection by *Listeria monocytogenes*, or *Staphylococcus aureus* [72], and the immune cells less effective at eradicating *S. aureus* and *S. pneumoniae* [69]. Additionally, and by modifying the gut flora using oral neomycin and ampicillin, a recent study investigated the association between the gut microbiota and prosthetic infection, and showed that a significantly greater proportion of animals with a disrupted gut microbiota (73%) developed an infection adjacent to a titanium tibial implant when compared with healthy controls (50%) [73]. Therefore, the microbiome offers a promising new target for new therapeutic approaches to regulate MSDs as well as in reducing susceptibility to infection [74–76].

1.3.5   Smoking

Cigarette smoking is one of the most prevalent and preventable risk factors for MSDs and orthopaedic surgery complications. Smoking rates among adults and teens are less than half of what they were in 1964; however, 42 million American adults and ~ 3 million middle and high school students continue to smoke. Cigarette smoke has over 7000 chemicals, 250 of which have been found to be toxic and at least 69 chemicals identified as carcinogenic. Smoking can damage nearly every organ in the body and has been identified as a risk factor for rheumatoid arthritis, osteoporosis, fracture, and lower back pain [77, 78]. Chronic wounds are a

significant and rising health problem that affect ~8.2 million people in the United States alone and ranging in treatment costs of $28.1 billion to $96.8 billion. The harmful effect of cigarette smoking on wound healing has been widely reported. Nicotine is a vasoconstrictor resulting in tissue ischemia and impaired tissue healing. It also delays wound healing by impairing oxidative metabolism and oxygen transport and by inducing platelet aggregation and subsequent microvascular occlusion, causing detrimental effects on microperfusion and, thus, tissue repair [79]. Postmenopausal women who smoke are a greater risk of bone fracture than women who never smoked [80], and smoking also has a significant and adverse effect on bone repair following injury. Smokers are 4.3 times more likely than nonsmokers to develop postoperative complications, such as infections, poor bone fusion, and delayed bone formation [81–84]. Smoking can delay fracture union, most notably in the tibial shaft, spine, foot, and ankle [85]. The risk of long-bone fracture nonunion is 12% higher in smokers than nonsmokers with a mean fracture healing time of 30.2 weeks in smokers versus 24.1 weeks in nonsmokers [86]. This may be due to the inhibitory effect of nicotine on osteoblast proliferation while inducing osteoclastic activity [87, 88]. Finally, smokers are more than twice as likely to develop an infection and 3.7 times likely to develop osteomyelitis following surgery [80].

1.4 Growth of the Orthopaedic Device Market

Successful healthcare depends on both prevention and curative intervention. Because prevention is rarely 100% effective, clinical services will always be needed. Although there is evidence that exercise therapy and psychosocial interventions are effective in relieving pain and improving function for many patients living with a MSD, as the symptoms and severity progress, orthopaedic surgery becomes inevitable. Surgical care can be remarkably cost-effective both in the short and longer term, even in comparison with nonsurgical interventions, and often brings immediate pain relief while restoring function to patients. This includes the insertion of a prosthesis that, for example, replaces the knee, hip, shoulder, ankle, or elbow or in the treatment of MSDs due to trauma, which will require use of devices including wires, pins, plates, and screws, for example. Between 1940 and 1975, a total of ~100 million metal prostheses were implanted into patients [89], and over more recent decades, trauma and arthroplasty surgical volumes have increased substantially. Given our aging population and longer life, this trend is only predicted to grow. In terms of total hip replacement (THR) and total knee replacement (TKR) surgery and in the United States alone, these procedures are projected to grow 171% (635,000 procedures) and 189% (1.28 million procedures) by 2030, respectively. Similarly, revision THR and TKR are projected to grow by 142% and 190%, respectively. By 2060, primary THR procedures are expected to reach 1.23 million (330% increase) and TKR, 2.6 million (382% increase). Similarly, revision THR is expected to reach 110,000 (219% increase) and revision TKR 253,000 (400% increase) by 2060. Females continue to make up the majority of patients (55–62%) [90].

In 2017, the global orthopaedic market was valued at $52.8 billion, and reported to be driven primarily by the growth of the aging population and the increased prevalence of diseases that affect the elderly, including osteoarthritis and osteoporosis. Presently, the market is primed to grow at a steady compound annual growth rate of 3.8%, to $66.2 billion in 2023. In 2017, the largest markets were in spinal, hip, and knee reconstruction implants and devices used in trauma fixation [91]. The most vital market for medical implant manufacturing is in the United States, where an annual revenue of ~$62 billion is generated. The European Union generates a revenue of ~$40 billion, followed by Japan, with a revenue of ~$20 billion [92].

Patient-specific implants and patient-specific instrumentation are expected to further drive growth in the hip and knee market. Building on the established success of hip and knee implants, use of reconstructive implants for the small joints such as the ankle, digits, elbow, shoulder, and wrist is currently experiencing high growth. This is considered to be due to the increasing awareness of patients and physicians to small joint options as well as the technological innovations that have contributed to more advanced implant designs. Minimally invasive and robotic surgical systems are also a growing trend as is the 3D printing of devices including spinal interbodies, craniomaxillofacial implants, and prosthetic devices, which will advance the physician's ability to provide customized solutions to patients.

1.5   Musculoskeletal Infection

Osteomyelitis, infected nonunions, septic arthritis, spondylodiscitis, hematogenous osteomyelitis, implant-associated infections, and necrotizing fasciitis are different manifestations of musculoskeletal infections. As the number of surgical procedures that manage and treat MSDs continues to rise, the threat of infection following surgery has increasingly significant clinical implications. Biomedical implants have revolutionized medicine, but they increase the infection risk. Infections are incapable of spontaneous healing and are often severe, life-threatening, and of high patient morbidity and are forecast to be one of the biggest healthcare challenges of the twenty-first century. Surgical procedures that require use of a prosthetic device are particularly exposed to the risk of infection, as when an implant is inserted into the body, the risk of infection increases 100,000-fold. At both the implant and tissue interface, human and planktonic bacterial cells compete for colonization of the surface and infections form due to adherence of bacteria and their subsequent biofilm formation. Around two-thirds of all human infections (both implant- and tissue-related) are believed to be complicated by biofilm. Biofilm-associated implant-related bone and joint infections, or biofilm-associated musculoskeletal infections, represent the worst complications of orthopaedic surgery and traumatology and are clinically important due to the extensive morbidity, cost of care, and socioeconomic burden that they cause [93, 94]. For example, an implant-associated infection is a devastating complication where patients often have to endure additional surgeries, lengthy exposure to systemic antibiotics, and potential permanent removal of the

implants or amputation. In 1–13% of patients, an implant-related infection is fatal [95]. Additionally, prosthetic infections are of a significant economic burden, and in United States, the estimated cost to treat an individual is about $50,000–$60,000 [96].

Yet, the rate and severity of septic conditions following orthopaedic surgery and the incidence of post-traumatic infections are projected to increase at a faster pace. The reasons for this are multifactorial and include a growing tendency to operate on high-risk patients, including geriatric patients, patients with diabetes, and patients who are immunocompromised or have comorbidities. As we live longer, the increased residency time of a prosthesis will provide continuous risk for infection during the implanted lifetime, this is of particular significance as implant devices are increasingly being used to treat the younger patient cohort [97]. Additionally, inefficient methods of diagnosing disease-causing pathogens combined with improvements needed in early detection methods are currently significant challenges, as is the rapid growth of antibiotic-resistant strains. The treatment of multi-drug resistant bacteria is extremely challenging and comprises a major public health concern as they continue to outpace the development of new antibiotics.

In recent decades, key discoveries have been made both within the clinical setting and in the scientific arena. For example, increased information and supporting data on the use of alternative treatments including debridement, antibiotics, irrigation, and retention of the prosthesis (DAIR) [98] have emerged, though its success varies from 15.8 to 75% [99], it may require multiple reoperations [100] and may be especially inferior in Gram-negative and drug-resistant infections [101]. The importance of the surrounding soft tissue envelope and the value of the free flap surgery have also improved surgical outcomes [102, 103]. From a scientific point of view, the discovery of biofilm formation [104] and the so-called race for the surface [105] have broadened our understanding of the pathogenesis of infection and led to the optimization of systemic antibiotics, and the identification of rifampin and fluoro-quinolones as antibiotics with anti-biofilm activity, for example [106]. The concept of the local application of antibiotics has been controversially discussed, but newer literature indicates some value [102] as well as more recent developments in the area of antimicrobial coatings and surface treatments of internal implants [107]. Despite these developments and improvements, currently, neither prophylaxis nor treatment is effective in all cases, and the outcome and the improvement of outcomes in musculoskeletal infections remains temperate. An additional complication is that there is often no consensus on optimal surgical or medical treatment strategies for many musculoskeletal infections, leading to uncertainty over best practice, which has resulted in a wide variation in individual practice.

In summary, current curative approaches often result in significant socioeconomic costs in addition to the risk of lifelong functional impairment for the patient or even death. The current challenge in treating infection is multifactorial, and combined efforts are required to improve prophylaxis, surgical treatment, antibiotic treatment, and rehabilitation in patients [108, 109]. As their incidence continues to rise, the treatment and care of patients following musculoskeletal infection is challenging, complicated, and costly and, as such, remains an important and unresolved problem.

1.6 Aim

The purpose of this book is to provide a modern and consolidated collection of evidence-based literature and clinical experience on the pathogenesis of problematic microorganisms and to also, where possible, interpret and resolve clinical "gold standards" in terms of both preventative and treatment orthopaedic strategies. The goal is to promote dissemination of scientific and clinical knowledge as well as determine current gaps in knowledge with the purpose of enhancing and advancing future discovery in the detection, prevention, and treatment of musculoskeletal infection.

References

1. D. Reynolds, L. Chambers, E. Badley, K. Bennett, C. Goldsmith, E. Jamieson, G. Torrance, P. Tugwell, Physical disability among Canadians reporting musculoskeletal diseases, The Journal of Rheumatology, 19 (1992) 1020.
2. T. Neogi, The epidemiology and impact of pain in osteoarthritis, Osteoarthritis and Cartilage, 21 (2013) 1145–1153.
3. D.B. Chaffin, G.B. Andersson, B.J. Martin, Occupational biomechanics, John wiley & sons, 2006.
4. A. Mäntyniemi, T. Oksanen, P. Salo, M. Virtanen, N. Sjösten, J. Pentti, M. Kivimäki, J. Vahtera, Job strain and the risk of disability pension due to musculoskeletal disorders, depression or coronary heart disease: A prospective cohort study of 69 842 employees, Occupational and Environmental Medicine, 69 (2012) 574–581.
5. E.M. Badley, I. Rasooly, G.K. Webster, Relative importance of musculoskeletal disorders as a cause of chronic health problems, disability, and health care utilization: Findings from the 1990 Ontario Health Survey, The Journal of Rheumatology, 21 (1994) 505–514.
6. W.T. Stauber, K.K. Knack, G.R. Miller, J.G. Grimmett, Fibrosis and intercellular collagen connections from four weeks of muscle strains, Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 19 (1996) 423–430.
7. A.E. Barr, M.F. Barbe, B.D. Clark, Work-related musculoskeletal disorders of the hand and wrist: Epidemiology, pathophysiology, and sensorimotor changes, Journal of Orthopaedic & Sports Physical Therapy, 34 (2004) 610–627.
8. Z. Jin, D. Wang, H. Zhang, J. Liang, X. Feng, J. Zhao, L. Sun. Incidence trend of five common musculoskeletal disorders from 1990 to 2017 at the global, regional and national level: results from the global burden of disease study 2017. Epidemics. 79(8): (2020)1014-1022. https://doi.org/10.1136/annrheumdis-2020-217050
9. S. Safiri, A.A. Kolahi, M. Cross, K. Carson-Chahhoud, A. Almasi-Hashiani, A. Ashrafi-Asgarabad, D. Hoy, G. Collins, A.D. Woolf, L. March, E. Smith. Global, regional and national burden of other musculoskeletal disorders 1990-2017: Results from the global burden of disease study 2017. Rheumatology 60 (2021):855–865.
10. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *GBD 2016 DALYs and HALE Collaborators. Lancet.* 2017 Sep 16*;* 390(10100)*:* 1260–1344.
11. USBJI (United States Bone and Joint Initiative). The burden of musculoskeletal diseases in the United States (BMUS). 3rd ed. Rosemont, IL: United States Bone and Joint Initiative; 2014a.

12. A.H. Mokdad, K. Ballestros, M. Echko, S. Glenn, H.E. Olsen, E. Mullany, A. Lee, A.R. Khan, A. Ahmadi, A.J. Ferrari, The state of US health, 1990-2016: Burden of diseases, injuries, and risk factors among US states, JAMA, 319 (2018) 1444–1472.
13. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Health Care Services; Committee on Identifying Disabling Medical Conditions Likely to Improve with Treatment. Selected Health Conditions and Likelihood of Improvement with Treatment. Washington (DC): National Academies Press (US); 2020 Apr 21. 5, Musculoskeletal Disorders. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559512/
14. A. Wasserman, Diagnosis and management of rheumatoid arthritis, American Family Physician, 84 (2011) 1245–1252.
15. CDC. Rheumatoid arthritis (RA). (2019a). [September 10, 2019]. https://www.cdc.gov/arthritis/basics/rheumatoidarthritis.html
16. Wright, N. C., Looker, A. C., Saag, K. G., Curtis, J. R., Delzell, E. S., Randall, S., and Dawson-Hughes, B. "The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine." Journal of Bone and Mineral Research, 2014. https://doi.org/10.1002/jbmr.2269.
17. Cooper, C., Campion, G., and Melton, L. J. "Hip fractures in the elderly: A world-wide projection." Osteoporosis International, 1992. https://doi.org/10.1007/BF01623184.
18. Harvey, N., Dennison, E., and Cooper, C. "Osteoporosis: Impact on health and economics." Nature Reviews Rheumatology, Vol. 6, No. 2, 2010, p. 99.
19. H. Brenner, W. Ahern, Sickness absence and early retirement on health grounds in the construction industry in Ireland, Occupational and Environmental Medicine, 57 (2000) 615–620.
20. Lombardi G, Ziemann E, Banfi G. Physical activity and bone health: What is the role of the immune system? A narrative review of the third way. Frontiers in Endocrinology 2019. https://doi.org/10.3389/fendo.2019.00060.
21. Sun F, Norman IJ, While AE: Physical activity in older people: A systematic review. BMC Public Health 2013; 13:449.
22. Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U; lancet physical activity series working group: Global physical activity levels: Surveillance progress, pitfalls, and prospects. Lancet 2012; 380:247–257.
23. Brown, W. J., Bauman, A. E., Bull, F. C., Burton, N. W. Development of evidence-based physical activity recommendations for adults (18–64 years). Report prepared for the Australian Government Department of Health. Final Report August 2012. Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/health-pubhlth-strateg-phys-act-guidelines/$File/DEB-PAR-Adults-18-64years.pdf
24. Schoenborn C, Adams P, Peregoy J. In: Statistics NCfH, Health behaviours of adults: United States, 2008–2010. Maryland: US Department of Health and Human Services; 2013.
25. Organisation WH. Prevalence of insufficient physical activity among adults. Global Health Observatory data repository. World Health Organisation. 2015. http://apps.who.int/gho/data/view.main.2487?lang=en. Accessed April 1, 2021.
26. Lewis R, Gomez Alvarez CB, Rayman M, Lanham-New S, Woolf A, Mobasheri A. Strategies for optimizing musculoskeletal health in the 21st century. BMC Musculoskeletal Disorders 20:164, 2019.
27. Ding D, Lawson KD, Kolbe-Alexander TL, Finkelstein EA, Katzmarzyk PT, van Mechelen W, Pratt M. Lancet physical activity series 2 executive C: The economic burden of physical inactivity: A global analysis of major noncommunicable diseases. Lancet 2016.
28. The Economic Cost of Physical Inactivity in Europe. In: Centre for Economics and Business Research. 2015. https://inactivity-time-bomb.nowwemove.com/report/
29. Schett G, Kleyer A, Perricone C, Sahinbegovic E, Iagnocco A, Zwerina J, Lorenzini R, Aschenbrenner F, Berenbaum F, D'Agostino MA, et al. Diabetes is an independent predictor for severe osteoarthritis: Results from a longitudinal cohort study. Diabetes Care 2013; 36(2):403–9.

30. Eymard F, Parsons C, Edwards MH, Petit-Dop F, Reginster JY, Bruyere O, Richette P, Cooper C, Chevalier X. Diabetes is a risk factor for knee osteoarthritis progression. Osteoarthritis and Cartilage 2015; 23(6):851–9.

31. McCarthy CJ, Mills PM, Pullen R, Richardson G, Hawkins N, Roberts CR, Silman AJ, Oldham JA. Supplementation of a home-based exercise programme with a class-based programme for people with osteoarthritis of the knees: a randomised controlled trial and health economic analysis. Health Technology Assessment. 2004; 8(46): iii–v 1–61.

32. Gordon R, Bloxham S. A Systematic Review of the Effects of Exercise and Physical Activity on Non-Specific Chronic Low Back Pain. Healthcare (Basel). 2016; 4(2).

33. Otero M, Esain I, Gonzalez-Suarez AM, Gil SM. The effectiveness of a basic exercise intervention to improve strength and balance in women with osteoporosis. Clinical Interventions in Aging 2017; 12:505–13.

34. R.J.F. Loos, A. Cecile, J.W. Janssens, Predicting polygenic obesity using genetic information, Cell Metabolism 25 (3) (2017) 535–543.

35. D. Yach, D. Stuckler, K.D. Brownell, Epidemiologic and economic consequences of the global epidemics of obesity and diabetes, Nature Medicine 12 (2006) 62–66.

36. World Health Organization. Fact sheet on obesity and overweight. 2015. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed April 1, 2021).

37. Ng, M.; Fleming, T.; Robinson, M.; Thomson, B.; Graetz, N.; Margono, C.; Mullany, E.C.; Biryukov, S.; Abbafati, C.; Abera, S.F.; et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014, 384, 766–781.

38. United Nations News Centre. https://www.un.org/sustainabledevelopment/blog/2016/01/report-governments-must-act-to-reverse-rise-in-childhood-obesity/, 2016 (accessed on April 1 2021).

39. Anderson K, Hamm RL. Factors that impair wound healing. J Am Coll Clin Wound Spec. 2012 Dec; 4(4):84–91.

40. Dening J. What's the connection between diabetes and wound healing? In: Butler N, ed. Healthline. 2017. https://www.healthline.com/health/diabetes/diabetes-and-wound-healing (accessed April 1, 2021).

41. E.A. Greco, R. Fornari, F. Rossi, V. Santiemma, G. Prossomariti, Is obesity protective for osteoporosis? Evaluation of bone mineral density in individuals with high body mass index International Journal of Clinical Practice 64 (2010) 817–820.

42. S. Gonnelli, C. Caffarelli, R. Nuti, Obesity and fracture risk, Clinical Cases in Mineral and Bone Metabolism 11 (2014) 9–14.

43. S. Haffner, H. Taegtmeyer. Epidemic obesity and the metabolic syndrome. Circulation. 108(13), 2003.

44. Rochlani Y, Pothineni NV, Kovelamudi S, Mehta JL. Metabolic syndrome: pathophysiology, management, and modulation by natural compounds. Therapeutic Advances in Cardiovascular Disease. 2017; 11(8):215–225. https://doi.org/10.1177/1753944717711379. Epub 2017 Jun 22. PMID: 28639538; PMCID: PMC5933580.

45. Barrón-Cabrera, E., González-Becerra, K., Rosales-Chávez, G. et al. Low-grade chronic inflammation is attenuated by exercise training in obese adults through down-regulation of ASC gene in peripheral blood: A pilot study. Genes & Nutrition 15, 15 (2020). https://doi.org/10.1186/s12263-020-00674-0.

46. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. Nature Reviews. Immunology. 2011; 11(11):738–49. https://doi.org/10.1038/nri3071. PMID: 21984069; PMCID: PMC3383854.

47. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators of Inflammation. 2010; 2010:289645. https://doi.org/10.1155/2010/289645. Epub 2010 Jul 14. PMID: 20706689; PMCID: PMC2913796.

48. Zhu M, Nikolajczyk BS. Immune cells link obesity-associated type 2 diabetes and periodontitis. Journal of Dental Research. 2014; 93(4):346–52. https://doi.org/10.1177/0022034513518943. Epub 2014 Jan 6. PMID: 24393706; PMCID: PMC3957341.

49. J. Feng, Q. Chen, F. Yu, Z. Wang, S. Chen, Z. Jin, Q. Cai, Y. Liu, J. He, Body mass index and risk of rheumatoid arthritis: A meta-analysis of observational studies, Medicine, 95 (2016).

50. A.D. Hanchate, A. Kapoor, J.N. Katz, D. McCormick, K.E. Lasser, C. Feng, M.G. Manze, N.R. Kressin, Massachusetts health reform and disparities in joint replacement use: difference in differences study, BMJ, 350 (2015) h440.

51. Daly RM. Exercise and nutritional approaches to prevent frail bones, falls and fractures: An update. Climacteric 2017; 20(2):119–24.

52. Cao Y, Winzenberg T, Nguo K, Lin J, Jones G, Ding C. Association between serum levels of 25-hydroxyvitamin D and osteoarthritis: A systematic review. Rheumatology (Oxford, England) 2013;52(7):1323–34.

53. Misra D, Booth SL, Tolstykh I, Felson DT, Nevitt MC, Lewis CE, Torner J, Neogi T. Vitamin K deficiency is associated with incident knee osteoarthritis. The American Journal of Medicine 2013; 126(3):243–8.

54. Molnar A, Jonasne Sztruhar I, Csontos AA, Ferencz C, Varbiro S, Szekacs B. Special nutrition intervention is required for muscle protective efficacy of physical exercise in elderly people at highest risk of sarcopenia. Physiol Int. 2016; 103(3):368–76.

55. R. Uauy, D.R. Hoffman, P. Peirano, et al., Essential fatty acids in visual and brain development, Lipids 36 (2001) 885–895.

56. L.R. DeChristopher, J. Uribarri, K.L. Tucker, Intake of high fructose corn syrup sweetened soft drinks, fruit drinks and apple juice is associated with prevalent coronary heart disease, in US adults, ages 45–59 y, BMC Nutrition, 3 (2017) 1–12.

57. D.J. Pattison, D.P. Symmons, M. Lunt, A. Welch, S.A. Bingham, N.E. Day, A.J. Silman, Dietary β-cryptoxanthin and inflammatory polyarthritis: Results from a population-based prospective study, The American Journal of Clinical Nutrition, 82 (2005) 451–455.

58. T. Barker, T.B. Martins, H.R. Hill, C.R. Kjeldsberg, R.H. Trawick, L.K. Weaver, M.G. Traber, Low vitamin D impairs strength recovery after anterior cruciate ligament surgery, Journal of Evidence-Based Complementary & Alternative Medicine, 16 (2011) 201–209.

59. Lin PH, Sermersheim M, Li H, Lee PHU, Steinberg SM, Ma J. Zinc in Wound Healing Modulation. *Nutrients*. 2017; 10(1):16. Published 2017 Dec 24. https://doi.org/10.3390/nu10010016.

60. Thompson KG, Audigé L, Arthur DG, Julian AF, Orr MB, McSporran KD, Wilson PR. Osteochondrosis associated with copper deficiency in young farmed red deer and wapiti x red deer hybrids. New Zealand Veterinary Journal 1994 Aug; 42(4):137–43. https://doi.org/10.1080/00480169.1994.35804.

61. Pedersini P, Turroni S, Villafane JH. Gut microbiota and physical activity: Is there an evidenced-based link? Sci Tot Environ. 727, 2020. https://doi.org/10.1016/j.scitotenv.2020.138648.

62. Hernandez CJ. The microbiome and bone and joint disease. Current Rheumatology Reports 2017; 19(12):77.

63. McCabe LR, Parameswaran N. Advances in probiotic regulation of bone and mineral metabolism. Calcified Tissue International 2018; 102(4):480–488.

64. Hernandez CJ. Musculoskeletal microbiology: The utility of the microbiome in orthopaedics. Journal of Orthopaedic Research 2021; 39(2):251–257.

65. Jansson P-A, Curiac D, Lazou AI. Probiotic treatment using a mix of three *Lactobacillus* strains for lumbar spine bone loss in postmenopausal women: A randomised, double-blind, placebo-controlled multicentre trial. Lancet Rheumatol. 2019; 1(3):e154-e162.

66. Ulici V, Kelley KL, Azcarate-Peril MA, Cleveland RJ, Sartor RB, Schwartz TA, Loeser RF. Osteoarthritis induced by destabilization of the medial meniscus is reduced in germ-free mice. Osteoarthritis and Cartilage. 2018 Aug; 26(8):1098–1109. https://doi.org/10.1016/j.joca.2018.05.016. Epub 2018 May 30. PMID: 29857156; PMCID: PMC7970023.

67. Schott EM, Farnsworth CW, Grier A, et al. Targeting the gut microbiome to treat the osteo-arthritis of obesity. *JCI Insight*. 2018; 3(8):e95997. Published 2018 Apr 19. https://doi.org/10.1172/jci.insight.95997.

68. Lahiri S, Kim H, Garcia-Perez I, Reza MM, Martin KA, Kundu P, Cox LM, Selkrig J, Posma JM, Zhang H, Padmanabhan P, Moret C, Gulyás B, Blaser MJ, Auwerx J, Holmes E, Nicholson J, Wahli W, Pettersson S. The gut microbiota influences skeletal muscle mass and function in mice. Science Translational Medicine. 2019; 11(502):eaan5662. https://doi.org/10.1126/scitranslmed.aan5662. 2019 Jul 24; PMID: 31341063; PMCID: PMC7501733.

69. Clarke TB, Davis KM, Lysenko ES, Zhou AY, Yu Y, Weiser JN. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nature Medicine 2010; 16(2):228–231.

70. Khosravi A, Mazmanian SK. Disruption of the gut microbiome as a risk factor for microbial infections. Current Opinion in Microbiology 2013; 16(2):221–227.

71. Libertucci J, Young VB. The role of the microbiota in infectious diseases. Nature Microbiology 2019; 4(1):35–45.

72. Khosravi A, Yanez A, Price JG. Gut microbiota promote hematopoiesis to control bacterial infection. Cell Host & Microbe. 2014; 15(3)"374–381.

73. Hernandez CJ, Yang X, Ji G, et al. Disruption of the gut microbiome increases the risk of periprosthetic joint infection in mice. Clinical Orthopaedics and Related Research 2019; 477(11):2588–2598. https://doi.org/10.1097/CORR.0000000000000851.

74. Ding, K., Hua, F., & Ding, W. (2020). Gut microbiome and osteoporosis. Aging and Disease, 11(2), 438–447. https://doi.org/10.14336/AD.2019.0523

75. Cheng S, Qi X, Ma M, Zhang L, Cheng B, Liang C, Liu L, Li P, Kafle OP, Wen Y, Zhang F. Assessing the relationship between gut microbiota and bone mineral density. Frontiers in Genetics 2020. https://doi.org/10.3389/fgene.2020.00006.

76. Li S, Mao Y, Zhou F, Yang H, Shi Q, Meng B. Gut microbiome and osteoporosis. Bone and Joint Res. 9(8). 2020. https://doi.org/10.1302/2046-3758.98.BJR-2020-0089.R1

77. Saag KG, Cerhan JR, Kolluri S, Ohashi K, Hunninghake GW, Schwartz DA. Cigarette smoking and rheumatoid arthritis severity. Annals of the Rheumatic Diseases 1997; 56(8):463–469. https://doi.org/10.1136/ard.56.8.463.

78. Green BN, Johnson CD, Snodgrass J, Smith M, Dunn AS. Association Between Smoking and Back Pain in a Cross-Section of Adult Americans. *Cureus*. 2016; 8(9):e806. Published 2016 Sep 26. https://doi.org/10.7759/cureus.80.

79. Whiteford L. Nicotine, CO and HCN: The detrimental effects of smoking on wound healing. British Journal of Community Nursing 2003 Dec*;* 8*(*12*):*S22–6*.*

80. Castillo RC, Bosse MJ, MacKenzie EJ, Patterson BM; LEAP Study Group. Impact of smoking on fracture healing and risk of complications in limb-threatening open tibia fractures. Journal of Orthopaedic Trauma 2005; 19(3):151–7. https://doi.org/10.1097/00005131-200503000-00001. PMID: 15758667.

81. Centers for Disease Control and Prevention. *Health effects of cigarette smoking*. 2018. https://www.cdc.gov/tobacco/data_statistics/fact_sheets/health_effects/effects_cig_smoking/index.htm

82. Bettin CC, et al. Cigarette smoking increases complication rate in forefoot surgery. Foot & Ankle International 2015; 36(5):488–93.

83. Martin CT, et al. The impact of current smoking and smoking cessation on short-term morbidity risk after lumbar spine surgery. Spine (Phila Pa 1976) 2016; 41(7):577–84.

84. Santiago-Torres J, et al. The effect of smoking on rotator cuff and glenoid labrum surgery: A systematic review. The American Journal of Sports Medicine 2015; 43(3):745–51.

85. Patel RA, et al. The effect of smoking on bone healing: A systematic review. Bone Joint Res 2013;2(6):102–11.

86. Scolaro JA, et al. Cigarette smoking increases complications following fracture: A systematic review. The Journal of Bone and Joint Surgery. American Volume 2014;96(8):674–81.

87. Rothem DE, Rothem L, Soudry M, Dahan A, Eliakim R. Nicotine modulates bone metabolism-associated gene expression in osteoblast cells. Journal of Bone and Mineral Metabolism 2009; 27(5):555–61.

88. Iqbal J, Sun L, Cao J, Yuen T, Lu P, Bab I, Leu NA, Srinivasan S, Wagage S, Hunter CA, Nebert DW, Zaidi M, Avadhani NG. Smoke carcinogens cause bone loss through the aryl hydrocarbon receptor and induction of Cyp1 enzymes. Proceedings of the National Academy of Sciences of the United States of America 2013 Jul 2; 110(27):11115–20.

89. B. Aksakal, Ö. Yildirim, H. Gul, Metallurgical failure analysis of various implant materials used in orthopaedic applications, Journal of Failure Analysis and Prevention, 4 (2004) 17–23.

90. Sloan M, Sheth NP. Projected volume of primary and revision total joint arthroplasty in the United States, 2030–2060. AAOS, 2018.

91. Global Data. Accessed on March 29, 2021. https://www.globaldata.com/

92. C. Azevedo, E. Hippert Jr, Failure analysis of surgical implants in Brazil, Engineering Failure Analysis, 9 (2002) 621–633.

93. Lamgni T. Epidemiology and burden of prosthetic join infections. J Antimicron Chemother. 69 (2014):i5-i10.

94. Kapadia BH, Berg RA, Daley JA, et al. Periprosthetic joint infection. Lancet 387 (2016):386–394.

95. G. Dickinson, A. Bisno, Infections associated with prosthetic devices: Clinical considerations, The International Journal of Artificial Organs, 16 (1993) 749–754.

96. A. Trampuz, W. Zimmerli, Prosthetic joint infections: Update in diagnosis and treatment, Swiss Medical Weekly, 135 (2005) 243–251.

97. Malizos KN. Global forum: The burden of bone and joint infections: A growing demand for more resources. The Journal of Bone and Joint Surgery. American Volume 2017 Mar 1; 99(5):e20. https://doi.org/10.2106/JBJS.16.00240.

98. Choi HR, von Knoch F, Zurakowski D, Nelson SB, Malchau H. Can implant retention be recommended for treatment of infected TKA? Clinical Orthopaedics and Related Research 2011; 469(4):961–969. https://doi.org/10.1007/s11999-010-1679-8.

99. Romanò CL, Manzi G, Logoluso N, Romanò D. Value of debridement and irrigation for the treatment of peri-prosthetic infections. A systematic review. *Hip International.* 2012; 22 Suppl 8:S19–24. https://doi.org/10.5301/hip.2012.9566.

100. Byren I, Bejon P, Atkins BL, et al. One hundred and twelve infected arthroplasties treated with 'DAIR' (debridement, antibiotics and implant retention): Antibiotic duration and outcome. The Journal of Antimicrobial Chemotherapy 2009; 63(6):1264–1271. https://doi.org/10.1093/jac/dkp107.

101. Papadopoulos A, Ribera A, Mavrogenis AF, et al. Multidrug-resistant and extensively drug-resistant Gram-negative prosthetic joint infections: Role of surgery and impact of colistin administration. International Journal of Antimicrobial Agents 2019; 53(3):294–301. https://doi.org/10.1016/j.ijantimicag.2018.10.018.

102. D. Lowenberg, M. Rupp, V. Alt. Understanding and treating chronic osteomyelitis. B. Browner, J. Jupiter, C. Krettek, P. Anderson (Eds.). Skeletal trauma: basic science, management and reconstruction (6th ed), Elsevier (2019). Chapter 25.

103. D. Pincus, J.P. Byrne, A.B. Nathens, A.N. Miller, P.R. Wolinsky, D. Wasserstein, et al. Delay in flap coverage past 7 days increases complications for open tibia fractures: A cohort study of 140 north American trauma centers. Journal of Orthopaedic Trauma 33 (2019):161–168.

104. J. W. Costerton, P.S. Stewart, E.P. Greenberg. Bacterial biofilms: A common cause of persistent infections. Science 284 (1999):1318–1322.

105. A.G. Gristina. Biomaterial-centered infection: Microbial adhesion versus tissue integration. Science 237 (1987):1588–1595.

106. W. Zimmerlia, P. Sendi. Role of rifampin against staphylococcal biofilm infections in citro, in animal models and in orthopaedic device related infections. Antimicrobial Agents and Chemotherapy. 63 (2019).

107. V. Alt. Antimicrobial coated implants in trauma and orthopaedics – A clinical review and risk-benefit analysis. Injury, 48 (2017), pp. 599–607.
108. V. Alt, PV Giannoudis. Musculoskeletal infections - A global burden and a new subsection in Injury. Injury. 50(12):2152–2153, 2019.
109. Moriarty TF, Kuehl R, Coenye T, et al. Orthopaedic device-related infection: current and future interventions for improved prevention and treatment. *EFORT Open Rev*. 2017; 1(4):89–99. Published 2017 Mar 13. https://doi.org/10.1302/2058-5241.1.000037

Chapter 2
Bacterial Adhesion, Virulence, and Biofilm Formation

Abinaya Sindu Pugazhendhi, Fei Wei, Megan Hughes, and Melanie Coathup

Abstract The adhesion of bacteria to implanted biomaterial and human tissue surfaces is the first and essential step in the pathogenesis of infection. Adhesion is followed by the formation of a biofilm barrier, which encases bacteria making them notoriously difficult to eliminate using conventional antimicrobial therapies. This chapter provides an overarching summary of some of the aspects involved in bacterial adhesion, virulence, and biofilm formation onto an abiotic or biotic surface. Although it is recognized that many independent species and strains, as well as the more complex polymicrobial infections, are associated with musculoskeletal infections, this chapter focuses on *Staphylococcus aureus*, a Gram-positive species commonly associated with orthopedic implant infection, and *Pseudomonas aeruginosa*, a Gram-negative species known to cause challenging soft tissue infections. We present contemporary knowledge of the host immune response to these species and the bacterial mechanisms used to manipulate the host innate and adaptive immunity responses, as well as describe the virulence factors produced, parameters that influence the adhesion of bacteria to surfaces, and, finally, the mechanisms involved in biofilm formation.

Keywords Bacteria · Bacterial virulence factors · Adhesion · Invasion · Biofilm · Bacterial proteins · Quorum sensing

A. S. Pugazhendhi · F. Wei · M. Coathup (✉)
Biionix Cluster & College of Medicine, University of Central Florida, Orlando, FL, USA
e-mail: melanie.coathup@ucf.edu

M. Hughes
School of Biosciences, Cardiff University, Cardiff, Wales, UK

2.1 Overview

In humans, most forms of bacteria reside within the body through two modes of growth: planktonic and biofilm. The former is the single-cell free-floating system which the host and conventional antibiotics can clear with ease. The latter is an accumulated biomass of bacteria with intercellular adhesion within an extracellular polysaccharide (EPS) matrix, a complex biofilm system highly resistant to medical treatment [1, 2]. For example, the minimal concentration of antibiotics required for the eradication of mature biofilm can be up to 100–1000 times higher than for planktonic bacteria [3]. The adhesion of bacteria to implanted biomaterial and human tissue surfaces is the first and essential step in the pathogenesis of infection. Human and bacterial cells compete for colonization of these surfaces, and if bacterial adhesion occurs before tissue regeneration takes place, then adhesion can ultimately lead to bacterial colonization and the development of peri-implant and tissue biofilm.

Bacterial communities on tissue and biomaterial surfaces present several challenges. First, these communities provide a reservoir of bacteria that can be shed into the body, facilitating the development of a chronic infection. Importantly, these bacteria can survive and remain dormant on the material surface for a relatively long period of time, and until the surrounding environment allows them to overgrow, such as in patients with decreased host immune activity or following poor tissue ingrowth to the prosthesis surface; a clinical infection then develops [4]. Second, and as mentioned above, those bacteria able to produce biofilm are highly resistant to treatment with antibiotics; therefore, once these bacterial communities form, they are extremely difficult to eliminate using conventional antimicrobial therapies. Finally, because host responses and antimicrobial therapies are often unable to eliminate bacteria growing in a biofilm, a chronic inflammatory response at the site of the biofilm may be produced, which can result in a severe loss of tissue structure and function [2, 5].

The scope of this area of research is vast and consists of a variety of bacterial species, each with their own processes, responses, and mechanistic behaviors. Therefore, this chapter is not exhaustive and instead aims to provide an overarching summary of some of the aspects involved in bacterial adhesion, virulence, and biofilm formation onto an abiotic or biotic surface. Although it is recognized that many independent species and strains, as well as the more complex polymicrobial infections, are associated with musculoskeletal infections, this chapter focuses on *Staphylococcus aureus*, a Gram-positive species commonly associated with orthopedic implant infection, and *Pseudomonas aeruginosa*, a Gram-negative species known to cause challenging soft tissue infections.

2.2 Host Response to the Insertion of a Non-phagocytosable Implant

A successful implant-associated infection ultimately involves complex interactions between the pathogen, the biomaterial, and the host immune response to both. Pathogens aside, the invasive nature of any surgery results in tissue injury and the generation of a niche of immune depression, a locus minoris resistentiae [6], where spontaneous clearance of planktonic bacteria does not take place, predisposing the implant to microbial colonization and infection [7, 8]. When coupled with the presence of a foreign body or implant and when in the absence of bacteria, the host will elicit an acute sterile immune-activated inflammatory response involving homeostatic mechanisms, tissue healing, and ideally fibrotic encapsulation to prevent further host responses (Fig. 2.1) [9]. The composition of the biomaterial itself determines the duration of the inflammatory response, and therefore, the severity and clinical manifestation of the implant-induced foreign body response is different and depending on the biomaterial used. Thus, the functional success of an implant is determined by the innate immune response and the transition and completion of the foreign body response.

On implantation and within seconds, serum proteins such as thrombin, fibrinogen, fibronectin, vitronectin, gamma globulin, albumin, and other immunomodulatory proteins are rapidly and spontaneously adsorbed and deadsorbed on the surface, instigating the formation of a thrombus [9–11]. Central to acute inflammation, activated platelets and endothelial cells release chemoattractants that recruit leukocytes, polymorphonuclear neutrophils (PMNs), and macrophages to the site of implantation. Recognition and activation by leukocytes and other immunocompetent human cells are dependent on surface receptor interactions of pattern recognition receptors (PRRs) expressed on these cells. PRRs are used to "sense" harmful situations, and the most studied of the five known families of PRRs are the toll-like receptors (TLRs), which are expressed both intracellularly and on the cell surface. PRRs also include C-type lectin receptors, retinoic acid-inducible gene I-like receptors, NOD-like receptors, and AIM2-like receptors [12]. Classically activated or "M1" macrophages together with the PMNs attempt to degrade the biomaterial and play significant roles in the ultimate transition to a foreign body response [13–15]. Initially, these cells attempt to destroy the foreign body through intrinsic mechanisms such as capture followed by phagocytosis and degranulation that delivers release of microbicidal proteolytic enzymes (e.g., neutrophil elastase), peptides (M-ficolin, lactoferrin, and peptidoglycan recognition protein), and reactive oxygen species (ROS), but the macrophages become "frustrated," and ultimately, cells exhaust their metabolic and phagocytic ability as the implant is too large to internalize [16, 17]. Similar to wound healing, the macrophages eventually transition into an "M2" phenotype, characterized by a reduced degradative capacity; secretion of anti-inflammatory cytokines, such as IL-10; and gained tissue remodeling functionality. During this complex process and in addition to the mechanisms of frustrated phagocytosis, overlapping events also result in adaptive leukocytes, such as

A. Tissue Injury and implantation of a biomaterial

Protein Adhesion, Adsorption/Deadsorption
e.g. thrombin, fibrinogen, fibronectin, vitronectin, gamma globulins, albumin

Inflammatory Cell Infiltration
(PMNs, monocytes, lymphocytes)

Tissue Response

Acute inflammation
PMNs, leukocytes, macrophages

Biomaterial composition
can prolong chronic inflammation

Chronic inflammation
e.g. monocytes, lymphocytes, mast cells, basophils
failed phagocytosis, cell exhaustion

IL-4, IL-13

Granulation tissue
Fibroblast proliferation & migration
Angiogenesis & capillary formation

Fibrous capsule formation
healing resolved

Biomaterial Response

Monocyte adhesion
Macrophage differentiation
Macrophage fusion

FBGC formation
Adhesion to biomaterial surface
Provides source of fibrogenic mediators
e.g. PDGF, VEGF, TGFβ leading to collagen deposition

B. Timeline until Fibrous Encapsulation

Time 0 · seconds · 1 day · 3 days · 1 - 2 weeks · 3 - 4 weeks

1. Implant inserted
2. Protein adsorption & deadsorption
3. Neutrophil interrogation
4. Macrophage interrogation
5. FBGC formation and adhesion
6. Fibrous capsule formation

Fig. 2.1 (**a**) The immune response following injury and the insertion of an implant and (**b**) the timeline involved during non-delayed healing

basophil, mast cell, and T cell recruitment to the site. These cells secrete IL-4 and IL-13 inducing foreign body giant cell (FBGC) formation in an attempt to increase their phagocytic functionality. While the exact mechanisms of fibroblast recruitment remain elusive, it has been suggested that biomaterial-adherent FBGCs serve as a constant source of fibrogenic mediators. Fibroblast-recruiting factors including platelet-derived growth factor, vascular endothelial growth factor, and transforming growth factor-ß are secreted by the FBGCs and result in fibroblast activation and collagen deposition, ultimately forming a capsule around the biomaterial to prevent further interaction with the host tissue [18–21]. Following fibrotic encapsulation, the inflammatory responses ultimately resolve, if no infection is present.

2.3 Bacteria Associated with Implant and Tissue Infection

2.3.1 Staphylococcus aureus

Staphylococcus aureus was included among the ESKAPE pathogens (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *and Enterobacter* species) recognized as the leading cause of antibiotic-resistant infections occurring worldwide in hospitals. *S. aureus* is a facultative anaerobe belonging to the genus *Staphylococcus* within the family of Staphylococcaceae. It is one of the most commonly identified clinically significant bacteria in a routine microbiology laboratory. The organism can cause superficial skin infections as well as life-threatening invasive diseases. *S. aureus* infections are initiated by the entrance of the microorganism through a breach of the skin or mucosa and can involve local structures or spread to distant organs to generate invasive infections such as bacteremia, pneumonia, infective endocarditis, musculoskeletal infections, as well as implant-associated infections. *S. aureus* infections can be very difficult to treat and, as a result, are often causes of significant morbidity and mortality. An important focus for the microbiology laboratory is the specific detection of methicillin-resistant *S. aureus* (MRSA), to identify colonized patients and subsequently implement institute infection control precautions [22]. The emergence and continuing spread of multiresistant *S. aureus* strains, such as MRSA and vancomycin-resistant *S. aureus*, complicate the treatment of staphylococcal infections and cause a significant economic burden. It is among the most frequently isolated bacterial pathogens in hospitals, and during the past decade, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains with high virulence have infected individuals without underlying risk factors [23, 24]. As such, treatment of staphylococcal infections has become increasingly difficult, and as described in 2.4, *S. aureus* has evolved several mechanisms to manipulate the innate and adaptive immunity responses.

2.3.2 Pseudomonas aeruginosa

The rod-shaped motile Gram-negative bacterium Pseudomonas aeruginosa is a ubiquitous organism that thrives in many environments, from soil, water, and animals to humans. In humans, it is an opportunistic pathogen that causes respiratory infections; superficial and deep cutaneous, urinary tract, and gastrointestinal infections; keratitis; otitis media; and bacteremia among others [25, 26]. However, serious infection frequently develops in immunocompromised patients, such as those undergoing chemotherapy or those with ecthyma gangrenosum and acquired immunodeficiency syndrome, in burn patients, and in patients with cystic fibrosis. It is the fourth most common cause of opportunistic nosocomial infections, accounting for approximately 10% of hospital-acquired infections, with case fatality due to bacteremia as high as

50%. In addition, its remarkable ability to develop resistance during antimicrobial treatment presents challenges and can complicate therapies aimed at eradicating both acute and chronic infections. As such and in 2017, *P. aeruginosa* was recognized as one of the most life-threatening bacteria and listed as priority pathogen for Research and Development of new antibiotics by the World Health Organization. Generally, infections are not caused by monospecies alone but rather colonization of a complex polymicrobial community. *P. aeruginosa* is often recognized as a co-colonizer along with other microbes such as *S. aureus*, Burkholderia cenocepacia, and Streptococcus parasanguinis. For example, *P. aeruginosa* and *S. aureus* often coinfect the lungs of patients with cystic fibrosis and in diabetic and chronic wounds. The natural resistance of *P. aeruginosa* to several antibiotic classes may be due to the organism's rapid ability to form biofilm, as well as due to the combination of low membrane permeability and active efflux pumps [27, 28]. Drug efflux is a key mechanism of resistance in bacteria as these systems pump solutes out of the cell. Thus, efflux pumps allow the bacteria to regulate their internal environment by removing toxic substances, including antimicrobial agents, metabolites, and quorum-sensing signals. The efflux systems involved in antibiotic resistance belong to the resistance-nodulation-division (RND) family [29]. When expressed, RND pumps confer clinically relevant levels of multidrug resistance and export a wide range of substrates. Four main efflux systems have been described to confer resistance to several antibiotics: MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM. These systems are composed of three proteins: (1) an efflux pump protein located in the cytoplasmic membrane (MexB, MexD, MexF, and MexY), (2) an outer membrane protein acting as a pore (OprM, OprJ, and OprN), and (3) a protein located in the periplasmic space that bridges the proteins located in the cytoplasmic and the outer membrane (MexA, MexC, MexE, and MexX) [30].

2.4 Immune and Cell Response to Bacteria

2.4.1 Immune and Bacterial Cell Response During Invasion

Interactions between commensal pathogens and host cells are critical for disease development. The host response is complex and involves the coordinated activity of a variety of cell types comprising both innate and adaptive immune systems. The innate response reacts to the pathogen via the recognition of a broad range of microbial determinants, and adaptive immune responses are triggered after a week of *S. aureus* infection [31]. The principle mechanism in antistaphylococcal host defense is opsonization with antibodies and complement proteins, followed by phagocytic clearance by macrophages and neutrophils. The presence of planktonic bacteria in the body causes the recruitment and infiltration of polymorphonuclear neutrophils, macrophages, dendritic cells, the gamma-delta subset of T lymphocytes, and natural killer cells to the infection site. Activated T cells subsequently

activate B cells, which differentiate into plasma cells, the producers of antigen-specific antibodies. A portion of these activated B cells become memory cells, which can be recalled to produce antibodies during reinfections. Unfortunately, in the case of persistent and chronic infection, adaptive memory responses are not always effective. These innate immune cells identify and phagocytose *S. aureus* via opsonins, which coat the bacterial cell surface with antibodies and complement. Following internalization, bacteria are eradicated through multiple mechanisms, including oxidative damage, enzymatic degradation, and antimicrobial peptide-induced lysis. *However, and as described in the sections below*, *bacteria including S. aureus* have evolved a myriad of immune evasion mechanisms to avoid clearance by the host. Most humans have high overall levels of antibodies against *S. aureus* as a consequence of preceding infections, but antibody titers differ strongly for specific antigens and are often not protective in immunocompromised patients, for reasons that are not clear.

An important defense function of PMNs against different pathogenic microorganisms (including not only bacteria but also viruses and fungi) is the production of the so-called neutrophil extracellular traps (NETs). First described by Brinkmann et al. [32] in 2004, NETs are comprised of a "network" of chromatin DNA and histones and are released in the extracellular milieu, following exposure to a number of inducing stimuli such as bacteria, fungal hyphae, inflammatory cytokines and chemokines, and immune complexes [33]. In addition to DNA, NETs are also composed of granule proteins, antimicrobial compounds such as antimicrobial peptides (AMPs), and proteases, these including, among others, lactoferrin, cathepsin G, neutrophil elastase, protease 3, and pentraxin 3 [34]. The granule proteins and DNA form extracellular fibers, and the nature of these NETs is able to "trap" pathogens and prevent spread of infection, as well as possessing bactericidal activity capable of binding and killing both Gram-positive and Gram-negative bacteria. However, the precise molecular mechanism of NET activity is presently unknown for most pathogens, and their involvement, if any, has not been detailed for implantable biomaterial-induced inflammation. The cationic AMPs are an important class of bactericidal agents. AMPs are expressed by host cells and are usually positively charged amphiphilic peptides that target the anionic surface of bacterial cell membranes. Due to this mechanism of action, they have been proven active against dormant cells within biofilms. For example, the peptide LL-37 has been reported to successfully clear *S. aureus* biofilm [35]. Further recent studies have demonstrated that certain AMPs, including LL-37, act synergistically with antimicrobial drugs eradicating biofilm bacteria, disrupting the biofilm matrix, or repressing biofilm production [36, 37].

Bacterial opsonization, complement activation, and release of pathogen-associated molecular patterns (PAMPs) trigger leukocyte chemokinesis and activation through the interaction with PRRs on the cell surface, alerting the immune system to the presence of invading microorganisms [38], as described above. This, in turn, results in the bacteria deploying its countermeasures including factors that inactivate and/or control the host humoral- and cell-mediated responses, and on strategies of persistence such as protective biofilm formation [39]. Bacteria are able

to not only suppress the bactericidal phagocytosis and release of cationic AMPs, enzymes, ROS, RNS, and NET action of leukocytes but release virulence factors while also hijacking and controlling the cellular machinery of these host cells, thereby altering the hosts immune defense response. Alternative bacterial strategies include evading neutrophil detection and phagocytosis through loss of flagella and motility, which is more frequently observed in *P. aeruginosa* mucoid colonies in patients with cystic fibrosis. The alginate exopolymeric matrix overproduced by mucoid *P. aeruginosa* cells is thought to have a protective function and is triggered when cells are in a relatively harsh environment, such as oxidative stress or during attack by the immune system. The overproduction of alginate provides additional protection from phagocytosis, and therefore in these situations, a bacterial defense mechanism is to convert from a non-mucoid to a mucoid phenotype [40, 41]. Furthermore, the decreased bactericidal activity of neutrophils after the exposure to biomaterial surfaces has been documented both in vitro [42] and in vivo [43], and has been correlated to severe biomaterial-related infections [44]. Finally, evidence suggests that incorporation of extracellular DNA (eDNA) and actin from necrotic neutrophils into the biofilm matrix protects the organisms from antimicrobial peptides and promotes biofilm maturation [45]. Therefore, tissue infection is a complex battle of host cells against the invading pathogen.

2.4.2   Bacterial Virulence Factors

2.4.2.1   *Staphylococcus aureus* Virulence Factors

A major contribution to the success of *S. aureus* as a pathogen is the plethora of virulence factors including secreted toxins (exotoxins; ~10% of the secretome) that manipulate the host's innate and adaptive immune responses, ensuring their survival [46, 47]. The immune-modulating virulence factors also include cofactors for activating host zymogens, and exoenzymes and all are strongly suspected to cause diseases such as toxic shock syndrome, staphylococcal scalded skin syndrome, necrotizing pneumonia, and deep-seated infections [48]. The main *S. aureus* toxins can be divided into three major groups—the pore-forming toxins (PFTs), serine proteases known as exfoliative toxins (ETs), and superantigens (SAgs). The pore-forming toxins act on the host cell membranes, resulting in inflammation and cytolysis of target cells. The ETs recognize and hydrolyze desmosome proteins in the skin and are associated with the loss of keratinocytes and cell-cell adhesion, inducing peeling of the skin and blister formation [49]. For example, ETs are the causative agents for staphylococcal scalded skin syndrome (SSSS), including Ritter's disease, toxic epidermal necrosis, bullous impetigo, and certain erythema cases. SSSS predominantly affects neonates, infants, and immunocompromised adult patients [50]. SAgs mediate massive cytokine production and trigger inflammation and T and B cell proliferation. Additionally, *S. aureus* is unique in its ability to coagulate blood through the production of multiple fibrinogen-binding proteins that

facilitate clumping. The formation of large, tightly packed clumps of cells has been demonstrated to be important for *S. aureus* virulence and immune evasion. These clumps are able to avoid detection by the host's immune system due to a fibrin(ogen) coat that acts as a shield, and the size of the clumps facilitates evasion of phagocytosis [51]. Coagulases and staphylokinases are bacterial cofactors able to hijack the host's coagulation system, while exoenzymes, including nucleases and proteases, cleave and inactivate various immune defense and surveillance molecules, such as complement factors, AMPs, and surface receptors that are important for leukocyte chemotaxis [46]. Collectively, these exotoxins modulate the host immune system and are critical for successful *S. aureus* infections.

Pore-forming toxins (PFTs) can be further divided into four types: (1) hemolysin-α (Hla or α-toxin), (2) γ-hemolysin, (3) leukotoxins (Luk) (e.g., LukED, LukSF (PVL), LukAB, LukMF', and LukPQ), and (4) phenol-soluble modulins (PSMs) (e.g., PSMα1–PSMα4, δ-toxin) [46]. These toxins are capable of damaging a wide range of human cell types, including epithelial cells, endothelial cells, T cells, erythrocytes, platelets, monocytes, macrophages, and certain leucocytes, either by degrading intercellular connections or by modulating immune responses [52, 53]. The role of α-toxin has been extensively studied. Hla or α-toxin is a pore-forming beta-barrel toxin, and one of the few *S. aureus* toxins that is core-encoded. α-Toxin is not only lethal on a cell and animal level but can also modulate cellular responses at sublytic concentrations. In brief, pore formation by α-toxin results in an influx of extracellular calcium into the cell, release of nitric oxide from endothelial and epithelial cells, the production of proinflammatory cytokines, pyroptosis of monocytes through the activation of caspase-1, and the production of NLRP3 inflammasomes [46, 54]. The pores also allow for the rapid release of ATP, K^+ ions, while also restricting the movement of macromolecules across the cell membrane [63]. Interestingly, α-toxin also upregulates host autophagy, allowing *S. aureus* to become tolerated by the host by downregulating expression of the toxin receptor, thus minimizing *S. aureus*-induced disease. For many years, α-toxin was thought to mediate cytolysis through nonspecific binding to the lipid bilayer of cells. However, this model did not explain the species specificity exhibited. It was not until Wilke et al. [55] identified the protein ADAM-10 (a disintegrin and metalloprotease 10) as the cellular receptor for the α-toxin receptor. ADAM-10, a zinc-dependent metalloprotease, binds to the surface of its target cell and initiates pore formation. Additionally, sublytic levels of α-toxin upregulate ADAM-10 expression resulting in the activation of the ADAM-10 protease, which cleaves the junction protein E-cadherin, resulting in disruption of the epithelial barrier in the skin [56]. Nanogram to microgram amounts of α-toxin can cause severe dermonecrosis when administered subcutaneously [57].

The bicomponent PFTs share structural homology with α-toxin, and have a similar pore formation mechanism; however, bicomponent PFTs primarily target leukocytes; thus, they are also known as leukocidins. Currently, five of the leukocidins are known to be associated with human infections: LukSF-PV (originally known as Panton-Valentine leukocidin, PVL), γ-hemolysins AB and CB (HlgAB, HlgCB), LukED, and LukAB (also known as LukHG) [58]. Two other bicomponent PFTs,

LukMF' and LukPQ, are associated with animal infections [59]. LukED is an important contributor to the virulence of *S. aureus* causing toxin-induced dermonecrosis of rabbit skin [60] and, when in microgram amounts, leads to acute lethality in mice [61]. γ-Hemolysins cause acute tissue injury and inflammation, and HlgAB has been shown to be required for *S. aureus* survival and proliferation during bloodstream infection, likely through macrophage evasion and nutrient (Fe^{2+}) release from erythrocytes [62]. The bicomponent toxin PVL is an important factor contributing to the epidemic spread and increased virulence of CA-MRSA strains. LukED targets cells of the adaptive immunity via CCR5, neutrophils, monocytes, and NK cells via CXCR1 and CXCR2, which also promote *S. aureus* pathogenesis [63]. In addition to their leukocidal activity, some leukocidins are able to lyse erythrocytes. Interestingly, bicomponent leukotoxin is the only factor known to enhance *S. aureus* survival as it plays a role in bacterial escape from phagocytes and neutrophils [64]. In additional to mediating cell lysis, many of the leukocidins have sublytic effects, causing extracellular Ca^{2+} influx on host cells and production of proinflammatory cytokines [65].

Phenol-soluble modulin (PSM) peptides belong to a family of amphipathic peptides uniquely found in staphylococci. PSMs have multiple roles in *S. aureus* pathogenesis, including cell lysis, biofilm formation, and immune modulation. PSMs can shape biofilms by forming channels needed for nutrient delivery and dissemination; however, the role of PSMs in extracellular cytolysis in vivo is unclear. In contrast, phagocytosed *S. aureus* produces PSMs to lyse neutrophils and osteoblasts intracellularly, and as such, the role of PSMs could be to mediate the intracellular escape of *S. aureus* [66]. This system of toxic protein production responds to a wide range of varying conditions, and understanding this mechanism will allow for a better control of staphylococcal infections. T cell SAgs represent the largest family of exotoxins produced by *S. aureus*. Due to their extreme stability and high toxicity in humans, some of them are classified as select agents for bioterrorism (i.e., staphylococcal enterotoxin B (SEB)) [46]. SAgs can be broadly divided into three groups, staphylococcal enterotoxins, staphylococcal enterotoxin-like superantigens, and toxic shock syndrome toxin-1. The primary role of these proteins appears to be immune evasion. SAgs are highly effective T cell mitogens that can stimulate up to 50% of T cells [67]. SAg-induced T cell proliferation is followed by a state of T cell anergy, where activated T cells failed to proliferate and/or undergo apoptosis. SAgs are one of the many ways *S. aureus* manipulates the host immune system to prevent the generation of functional adaptive immunity. Staphylococcal protein A (SpA) is the only known B cell superantigen produced by *S. aureus*. During intravenous infection, SpA prevents opsonophagocytosis of the bacteria by binding to immunoglobulins and impedes the development of specific anti-*S. aureus* antibodies [46, 68].

More recently, the formation of extracellular vesicles (EVs) has been shown to play an important role in bacterial virulence and pathogenesis. EVs are nano-sized (20–500 nm), spherical, bilayered membrane vesicles which are sometimes associated with filamentous structures known as nanopods or nanotubes. Bacterial EVs package diverse proteins and influence the host-pathogen interaction, but the mechanism of EV biogenesis remains poorly understood; however, they are increasingly

being recognized as important mediators through their transfer of a wide variety of molecular cargoes. EVs were first observed in the 1960s, and bacteria secrete what are now referred to as outer membrane vesicles (OMVs). OMVs likely play important roles in bacterial pathogenesis due to the transport of multiple virulence factors able to serve as immune modulators [69]. For example, *Staphylococcus aureus* generates and releases OMVs that package cytosolic, cell wall-associated, and membrane proteins, as well as glycopolymers and exoproteins, including α-hemolysin, leukocidins, phenol-soluble modulins, superantigens, and enzymes, thus representing a secretory pathway that allows cell-free intercellular communication [70]. Additionally, OMV production may serve as a mechanism for *S. aureus* to transport toxins and other components of its secretome into host cells while also protecting the contents of the OMV lumen from degradation or neutralization. Moreover, toxin-positive *S. aureus* OMVs elicit skin barrier disruption in mice with characteristic atopic dermatitis-like skin inflammation [71, 72]. Outer membrane vesicle generation has been reported to occur following exposure to environmental stressors, such as antibiotics, oxidative stress, iron depletion, and lateral gene transfer (via RNA or DNA), suggesting that OMV generation may represent an adaptive mechanism for *S. aureus* growth while in a hostile host environment [73].

2.4.2.2 *Pseudomonas aeruginosa* Virulence Factors

Pseudomonas aeruginosa possesses an impressive arsenal of virulence factors to initiate infection and persistence in the host. These include secreted factors, such as elastase, protease, surface expression of ferripyochelin-binding protein, phospholipase C, hydrogen cyanide, exotoxin A (ExoA), and exoenzyme S (ExoS), as well as cell-associated factors, such as lipopolysaccharide, flagella, and pili [74]. The expression of these factors is tightly regulated. Five protein secretion systems have been identified in *P. aeruginosa* (types I, II, III, V, and VI), and each has different functions [75]. The type II system (T2SS) is sometimes referred to as the "general secretion pathway" and promotes the outer membrane translocation of large (including some multimeric) exoproteins that are already folded in the periplasm [76]. ExoA is a highly toxic virulence factor released by *P. aeruginosa* into the extracellular medium via the T2SS. ExoA decreased transepithelial resistance and enhanced paracellular permeability of type II pneumocyte cultures on permeant filters, indicating altered epithelial integrity [77]. Most strains of *P. aeruginosa* produce virulence factor exotoxins that inject directly into the cytoplasm of target cells using a syringe-like apparatus common to many Gram-negative pathogenic bacteria. A needle-like complex of proteins injects toxic proteins, called effectors, directly into the cytosol of host cells, as controlled by the type 3 secretion system (T3SS) [78, 79]. To date, four effectors of *P. aeruginosa* produced by the T3SS have been identified, these are ExoS and exotoxin T (ExoT) (bifunctional toxins with amino-terminal GTPase-activating proteins activity and carboxy-terminal adenosine diphosphate ribosyl transferase activity), exotoxin U (ExoU, a phospholipase), and exotoxin Y (ExoY, adenylate cyclase) [80]. Once in the cytoplasm of host epithelial cells,

exotoxins from the T3SS induce cell death by necrosis or apoptosis, thus favoring disruption of epithelial barriers [79]. Such persistent survival of *P. aeruginosa* also likely alters epithelial integrity and repair processes, by interacting with intracellular proteins and/or structures [80]. The major phylogenetic groups (I and II) differ in their type III systems. The majority of strains encode either ExoS (group I) or ExoU (group II) with different associated toxins, and this impacts epithelial cell invasion and/or cytotoxicity. It has been shown that *P. aeruginosa* strains that produce ExoS but not ExoU can invade and survive within epithelial cells. Most recently, the type VI system (T6SS) has been described. This system functions by "stabbing" other *P. aeruginosa* cells resulting in cell death and has been proposed to be used for intra-strain competition [81]. *P. aeruginosa* also produces a range of bacteriocins termed pyocins (S, R, and F types), which kill other sensitive strains of *P. aeruginosa* and are also thought to be used for intra-strain competition [82–84].

The intricate biological process of cutaneous wound healing is achieved through precise and highly programmed events, and infections with *P. aeruginosa* can result in the failure of these injuries to heal, thus becoming chronic wounds [85, 86]. Dermal fibroblasts and keratinocytes play a significant role in the process of reepithelialization during wound healing, and *P. aeruginosa* delays the proliferative phase of wound repair through the release of virulence factors that lead to reduced or loss of lamellipodial structures, stress fibers, focal adhesions, and destruction of the actin cytoskeleton which results in an alteration in the cell morphology within the cutaneous layer [83, 87, 88]. Additionally, several studies have reported that *P. aeruginosa* infection is associated with the disruption of cell-cell contacts and loss of cellular junctions [89]. Rhamnolipids are a class of glycolipid produced by *P. aeruginosa*, among other organisms, and are frequently cited as bacterial surfactants. *P. aeruginosa* is the most competent producer of rhamnolipids, which contribute to epithelial barrier disruption through tight-junction-associated alterations. For example, *P. aeruginosa* elastase promotes collagen degradation by inducing the conversion of the inactive precursors of several MMPs into active enzymes, altering the extracellular matrix of cutaneous, airway, and corneal epithelia through degradation of type I and type IV collagen proteins [85]. *P. aeruginosa* is known to strongly adhere both to desquamated bronchial epithelial cells and to the underlying basal lamina, which is mainly composed of laminin, which have heparin-binding domains that interact with cell-surface-bound heparan sulfate. In human skin wounds, it has been shown that *P. aeruginosa* was able to colonize the upper epidermal layers before invasion into the dermis, causing a loss of epidermis and de-keratinization of skin constructs, as well as partial loss of the basement membrane [90].

2.4.3   Bacterial Invasion of Host Cells

Arguably, most research on musculoskeletal infections has centered on *S. aureus* due to its frequency, plasticity, and resistance. As has been described, *S. aureus* possesses a vast repertoire of virulence and immune evasion factors that facilitates its

dual lifestyle as either a commensal or a pathogen. More importantly, *S. aureus* displays a complex regulatory network, composed of a number of genes, many of which remain unknown, but which allow the cross talk between regulators, thus permitting this species to rapidly switch on and off virulence factors to adapt to and survive in changing microenvironments [91]. *S. aureus* has been traditionally considered as an extracellular pathogen; however, it has been shown to evade both antibiotics and host defenses by hiding in non-phagocytic and phagocytic host cells [92]. Up to 8% of *Staphylococcus aureus* species are able to invade non-phagocytic cells such as osteoblasts within 0.5 hours [93] and 2 hours [94] of exposure, where one *S. aureus* per osteoblast was sufficient to induce the death of approximately 10% of the osteoblast population within 2 hours, and 70% within 8 hours of inoculation in vitro [93]. *S. aureus* is internalized through binding of fibronectin-binding proteins located on the surface of *S. aureus*, with fibronectins found on the osteoblast surface and connected to the integrin dimer $\alpha_5\beta_1$ molecule [95]. Although host cell internalization allows *S. aureus* to evade antibiotics that are inactive intracellularly, as well as avoid interaction with activated professional phagocytes, it has nevertheless been reported that *S. aureus* may also survive phagocytosis by both neutrophils and macrophages, remaining within these cells for up to 5 and 7 days [93]. Although the proportion of *S. aureus* has been shown to be higher in macrophages than osteoblasts (100-fold), the proportion that survived within the osteoblast was significantly higher [96, 97]. This may equally apply to other nonprofessional phagocytes and due to their inability to effectively destroy bacteria when compared to the proteolytic enzyme activity offered by professional phagocytes such as the macrophage.

Osteoblast invasion and persistence of *S. aureus* are known to contribute to the pathogenesis of osteomyelitis [94, 98, 99]. An interesting study by Hamza et al. [99] showed that when rat osteoblasts were infected with *S. aureus* ex vivo, and then administered to rats in vivo, the intracellular bacteria could initiate infection of open fractures. Of note, clinical isolates from other staphylococcal species, such as *S. epidermidis*, show much lower invasion rates. At the same time, these surviving internalized bacteria and a subset of bacteria in biofilm have been shown to adopt a small colony variant-like phenotype (SCV) characterized by slow-growth kinetics and low levels of cytotoxic factor secretion, enabling their survival over long periods of time [100–102]. Compared with wild-type bacteria, SCVs have demonstrated higher intracellular persistence and lower sensitivity to antibiotics, which may be associated with their lower cytotoxicity [96, 103]. The transmembrane potential of bacteria is critical to the uptake of positively charged particles, such as AMPs and antibiotics; however, when wild-type bacteria were converted into SCVs, the membrane potential reduced. This change could indirectly reduce the bactericidal activity of antimicrobial agents [104]. In addition, and upon exiting the original host cells, SCVs are able to rapidly revert to the wild type, and demonstrate a highly toxic, invasive phenotype, thereby infecting new cells. This may lend to explaining why, for example, chronic osteomyelitis patients develop repeated infections. Host cell death has been reported to be due, in part, to an *S. aureus*-induced increase in the intracellular levels of ROS and hydrogen peroxide. It is noteworthy that, besides

the significant changes in ROS, *S. aureus* internalization into osteoblasts also led to significantly higher production of IL-6 and IL-12, macrophage chemoattractant protein 1, IL-8, IP-10, RANTES, and RANK-L, and prostaglandin E_2 (important cytokines able to promote osteoclastogenesis and bone resorption) [105, 106], as well as decreased alkaline phosphatase expression [93]. Thus, internalized bacteria induce decreased cell activity followed by apoptosis and/or host cell necrosis prior to colonizing the implant or tissue surface [107]. Moreover, osteoclasts are activated, bone resorption increases and bone homeostasis is disrupted [96].

A recently discovered mechanism of immune invasion and persistence is bacterial invasion within the submicron-sized interconnected lacunae-canaliculi porous system of bone [108]. This study showed that *S. aureus* is capable of colonizing and proliferating within this system, requiring them to deform to sizes as small as 0.2 μm. Further, BrdU immunoelectron microscopy was used to confirm that the bacterial cells at the leading edge of invasion were actively proliferating, as opposed to persisting in a dormant state. This finding was surprising given that *S. aureus* is considered a nonmotile bacterium with no known mechanisms of active translocation. This is of concern as bacteria within the confined geometries of canaliculi or lacunae are protected from immune cell attack, and it is possible that the bacteria could survive for years by dissolving the adjacent bone mineral matrix as a source of nutrients. The extent of bacterial invasion within this porous network is not known but could be a major factor in the failure of surgical debridement of infected bone.

2.5 Bacterial Adhesion to Surfaces

The concept "race to the surface" has been introduced to describe the competition between host cells and bacteria to adhere, replicate, and colonize an implant surface [7]. The rapid integration of an implant within host tissue is essential for its success, and there is evidence that immediate integration is also crucial for preventing bacterial adhesion and colonization. If the host cells win the race, a stable interface between cells and the implant surface is formed; however, if bacterial adhesion occurs before tissue repair takes place, often host defenses cannot prevent surface colonization and biofilm formation [2, 109]. The bacterial surface is a highly specialized organelle, and one of its key purposes is to facilitate adherence. Changes in response to surface engagement are far-reaching and can affect bacterial metabolism, respiration, and regulation of colonization- and virulence-specific genes [109]. The implantation of a foreign device provides the ideal platform for the adherence of pathogens; however, the molecular and physical interactions that govern bacterial adhesion to biomaterials are not well understood. Bacterial adhesion to a surface is considered an extremely complex process influenced by *environmental factors* (serum proteins, flow conditions, temperature, bacterial concentration, time of exposure, antibiotics), *bacterial factors* (Gram-positive or Gram-negative, surface energy and charge, outer membrane molecular receptor expression), and *material*
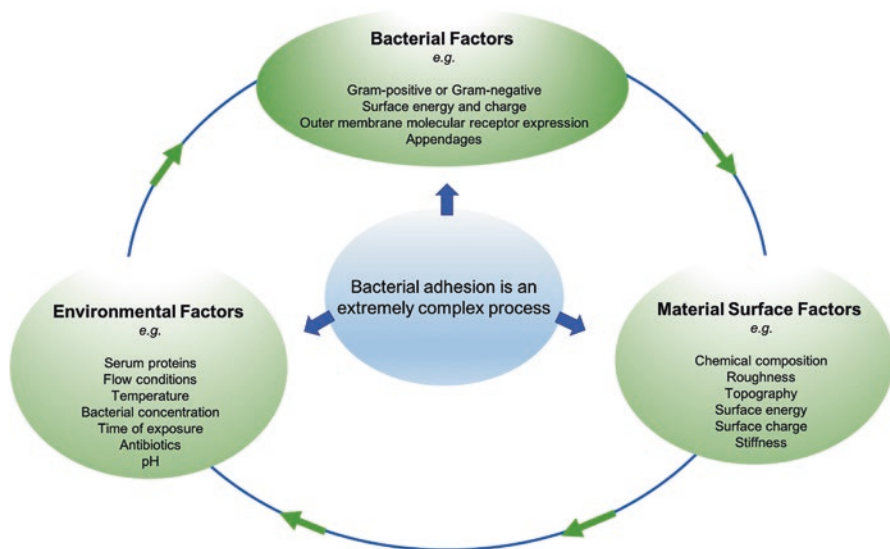
Fig. 2.2 Bacterial adhesion is an extremely complex process involving environmental, bacterial, and material surface factors; all are interconnected

surface factors (chemical composition, roughness, topography, surface energy, charge, etc.) (Fig. 2.2). The properties of each factor are interconnected, thus introducing much complexity. Fluid flow conditions are considered a dominant factor that strongly influences the number of attached bacteria as well as the subsequent biofilm structure and performance. It is generally considered that higher shear rates result in higher detachment forces that result in decreasing the number of attached bacteria, while they make the biofilm denser and thinner. Surface roughness, texture, and wettability are regarded as the most significant surface factors; however, the configurations required to encourage host cell adhesion and proliferation can also provide advantageous conditions for pathogen growth [110]. For example, and in terms of osseous integration with an implant surface, an increased surface roughness correlates with faster and firmer integration into the surrounding bone tissue. However, the majority of bacterial studies indicate a positive correlation between increased surface roughness and the quantity of adhering bacteria [110, 111].

Generally, bacteria prefer to grow on available surfaces rather than in the surrounding aqueous phase. Motility is central to a number of bacterial behaviors such as biofilm formation, virulence, and host colonization. Bacteria are able to move and colonize surfaces using energy-dependent cellular mechanisms whereby the bacteria can directly and actively control where they move to using flagella, for example (motile) or passive movement, which relies on the environment and the associated forces resulting in random and limited motion (nonmotile). Active motility includes swimming, swarming, twitching, and gliding, although the mechanism of movement differs and remains to be fully elucidated in many bacterial species. Swimming is dependent on flagella, swarming occurs when groups of

hyper-flagellated bacteria move together across surfaces, twitching is dependent on the extension and retraction of type IV pili, and gliding is the continuous, smooth movement of the bacteria, either individually or in groups either in a linear fashion or in whirls. *P. aeruginosa* is flagellated and a motile organism, while *S. aureus* has historically been regarded as nonmotile organism, but recently was shown to move over soft agar through spreading dendrites and aided by the production of PSMs [112].

Bacterial adhesion begins through the initial attraction of cells to the surface followed by their adsorption and subsequent attachment. Adhesion can be described as a two-phase process including an initial, instantaneous, unspecific, and reversible physical phase (phase one) and a time-dependent, specific, and irreversible molecular and cellular phase (phase two) [2, 4]. In phase one, and from an overall physicochemical viewpoint, bacterial adhesion can be mediated by both long-range (>50 nm) and nonspecific interaction forces, as well as specific forces that act in highly localized regions of the surface, and over distances <5 nm. The fluidic behavior of many bacteria during interactions and adhesion to abiotic and biotic surfaces are regarded as colloids because the bacterial cell-surface properties, e.g., size of 0.5–2 μm and shape, typically resemble those of colloids [113]. The planktonic bacteria in bulk fluid are freely suspended before attaching to the surface, and long-range interactions consist of bacteria moving to or are moved to a material surface through the effects of physical forces, such as flow of the fluid (Brownian motion) and mass transport processes such as fluid convection, diffusion, and sedimentation or via van der Waals attraction forces, gravitational forces, surface electrostatic charge, and hydrophobic interactions. Bacterial movement can also be directed by concentration gradients of diffusible ("chemotaxis") or surface-bound ("haptotaxis") chemical factors referred to as chemoattractants (e.g., amino acids, sugars, oligopeptides). These short-range interactions consist of establishing chemical bonds, ionic, dipole, and hydrophobic interactions. Chemotaxis occurs in almost all microbes and can modulate bacterial growth on surfaces by regulating cellular adhesion components and preparing cells for cell-cell and cell-surface interactions [4]. Bacteria are transported to the surface via long-range interactions, and when in closer contact, short-range interactions become more influential. Both specific and nonspecific interactions may play an important role in the ability of the cell to attach to (or to resist detachment from) the biomaterial surface [2, 4, 114]. In phase two, molecular-specific reactions between bacterial surface structures and the material become predominant. Once microorganisms reach the proximity of a surface, attachment is determined by physical and chemical interactions, which may be attractive or repulsive, depending upon the complex interplay of the chemistries of the bacterial and biomaterial surfaces, and the aqueous phase [4]. Initial adhesion to abiotic biomaterial surfaces is generally unspecific. Adhesions are the surface receptors responsible for bacterial adhesion, and bacteria may possess multiple and different adhesins that are applicable for different surfaces [114, 115]. In addition to adhesins, adhesion also occurs through bacterial surface polymeric filamentous cell appendages, including capsules, fimbriae, pili, and pilus-like adhesive structures [116]. Beyond phase two, irreversible attachment is facilitated in bacterial strains
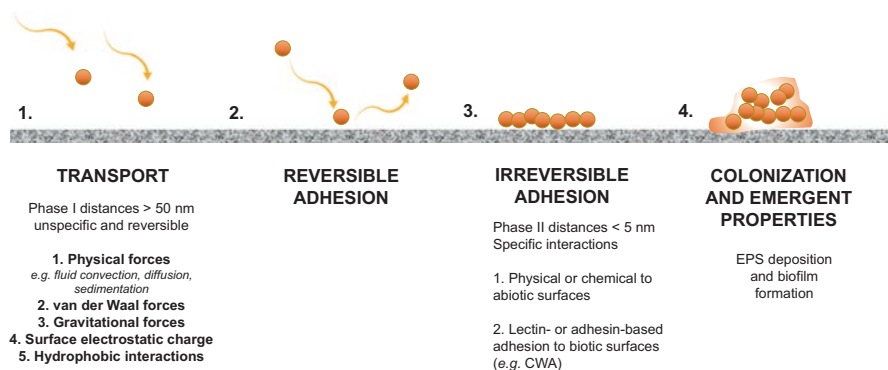
1. TRANSPORT

Phase I distances > 50 nm
unspecific and reversible

1. Physical forces
e.g. fluid convection, diffusion, sedimentation
2. van der Waal forces
3. Gravitational forces
4. Surface electrostatic charge
5. Hydrophobic interactions

2. REVERSIBLE ADHESION

3. IRREVERSIBLE ADHESION

Phase II distances < 5 nm
Specific interactions

1. Physical or chemical to abiotic surfaces

2. Lectin- or adhesin-based adhesion to biotic surfaces (*e.g.* CWA)

4. COLONIZATION AND EMERGENT PROPERTIES

EPS deposition and biofilm formation

Fig. 2.3 Initial bacterial (cocci) adhesion to a surface

able to secrete EPS and form biofilm, if provided with an appropriate supply of nutrients (Fig. 2.3).

In contrast, bacterial adhesion to host tissues can be highly specific in the initial phase. *S. aureus* carries a wealth of pathogenic factors, which include not only the secreted exoenzymes and toxins that promote tissue damage and distant diseases as described in the sections above but also surface adhesins that promote cell and tissue colonization. Bacterial adhesion to extracellular matrix (ECM) molecules has been thoroughly reviewed [117], and *S. aureus* possesses a rich repertoire of adhesins, including cell wall-anchored (CWA) microbial surface components that recognize adhesive matrix molecules, as well as a secretable expanded repertoire of adhesive molecules that further mediate adhesion to ECM proteins [118]. Cell wall-anchored surface proteins are multifunctional, and their activities include not only adhesion to host cells and tissues but also in the invasion of non-phagocytic cells, biofilm formation, and evasion of host immune responses. *S. aureus* can express up to 24 distinct CWA proteins, which are covalently bound to the cell wall peptidoglycan, by transpeptidases known as sortases [97, 119]. CWA proteins are known to be of crucial importance in the interaction of *S. aureus* with its host both in the commensal state and during infection; however, for the most part, there is a lack of understanding of how these CWA proteins interact with immune pathways, and their capacity to activate T cells also remains to be fully established. Based on molecular structure and its arrangement, the CWA proteins of *S. aureus* have been classified into four families, the microbial surface components recognizing adhesive matrix molecule (MSCRAMM) family, the near iron transport (NEAT) motif family, the three-helical bundle family, and the G_5-E repeat family. *S. epidermidis* and *S. aureus* express dozens of MSCRAMMs that have the capacity to bind to human matrix proteins such as collagen, fibronectin, vitronectin, elastin, prothrombin, von Willebrand factor, and fibrinogen (Fg) [120]. Bacteria also possess modular adhesins that engage multiple surface receptors, often in a cooperative manner, giving rise to extremely high binding avidity. Examples include fibronectin-binding protein A, which binds to fibronectin by forming a ß-zipper [121]. This zipper-like mechanism

guarantees a productive interaction between bacterium and host cell and often initiates bacterial uptake by non-phagocytic cells. Another even more immediate way to trigger uptake by using a zipper-like mechanism is exemplified by the *Pseudomonas aeruginosa* surface lectin LecA, which forms a direct zipper with the host lipid membrane by binding the glycosphingolipid Gb3, thereby triggering membrane bending and facilitating invasion [109, 122]. The contribution of Fg and fibronectin binding in disease progression is thought to be critical. *S. aureus* clumping factor A (ClfA) is a major staphylococcal adhesin and a major Fg binding protein. As such, ClfA mediates staphylococcal binding to immobilized Fg- or fibrin-coated surfaces, promoting bacterial adherence to tissues, blood clots, as well as abiotic biomaterial surfaces [123, 124]. Of note, CWA proteins also play a role in immune cell evasion. *S. aureus* produces three proteins that interfere with immunoglobulin (Ig) deposition. The best known is CWA protein A (Spa). Protein A binds to the Fc fragment of IgG and to the Fab portion of V_H3-type B cell receptors, producing a "camouflage coat" of nonspecific Igs on the *S. aureus* surface, preventing receptor-mediated phagocytosis and resulting in bacterial evasion of the host immune response [125, 126]. These examples of CWA proteins are by far not exhaustive, but should serve to demonstrate that although bacterial adhesins are uniquely adapted to accomplish colonization of a specific niche, the mechanisms underpinning their function are also well conserved across bacterial species. Of note, CWA proteins have been proposed as therapeutic targets for the generation of vaccines.

2.5.1   Environmental Factors

Environmental factors including fluid flow conditions, temperature, exposure time, bacterial concentration, chemical treatment, and the presence of antibiotics affect bacterial adhesion [2, 4, 114]. A primary factor that determines adhesion is fluid flow adjacent to the surface. Taking the simplest case of ligand-/receptor-mediated attachment, the number of bonds that can form will be a function of ligand and receptor densities. If each bond requires a specific force to break it, the number of bonds between bacterium and surface will determine the shear stress that the attached bacterium will be able to resist. As such, decreased bacterial adhesion at higher flow rates has been clearly established, where a higher shear force correlates with a higher bacterial detachment rate. Katsikogianni et al. [127] investigated bacterial adhesion to several substrate compositions and showed a correlation as the number of adherent bacteria significantly decreased with an increasing shear force from 150 s^{-1} to 1500 s^{-1}. However, it is also important to note that there is an optimal flow rate that encourages bacterial adhesion to a surface, and therefore, there is a balance between the rate of delivery and the force acting on the bacteria [128]. For example, Mohamed et al. [129] reported that in the case of a higher number of receptors/cells, *S. aureus* adhesion to collagen-coated coverslips increased at shear rates between 50 and 300 s^{-1} and only decreased at rates above 500 s^{-1}. On the contrary, a low level of bacterial concentration on a surface can also initiate a host

immunomodulatory response, thus preventing the infection. This has been termed the "implant infection paradox," where a competent immune system in addition to the ability of local tissue to adhere, spread, and grow in the presence of a low concentration of bacteria is able to prevent bacterial colonization. In contrast, an intolerant immune system with an inability to form a robust local tissue response renders the implant surface more susceptible to invasion [130]. For example, *Staphylococcus* spp. have been shown to adhere to medical implants in vitro at an inoculum size of <10 bacteria; however, this was likely to be insufficient for robust biofilm establishment in vivo due to the "race for the surface" theory of competitive colonization in immunocompetent individuals [131–133]. However, when in higher numbers, bacterial concentration is positively correlated with surface adhesion, with higher concentrations of bacteria being associated with a greater degree of implant surface coverage [131, 132].

In addition to the role of hydrophobic interactions on a surface, Lewis acid-base interactions also facilitate bacterial adhesion. As such, the optimum pH of both *P. aeruginosa and S. aureus* is weakly acidic, falling in the range of pH 4–6, with deviations from these values resulting in decreased adhesion to substrata [134, 135]. *Pseudomonas* spp. showed optimal adhesion to medical-grade titanium at pH 6, the point of zero charge, due to the overall lack of both attractive and repulsive electrostatic forces [134]. A growing body of evidence suggests that eDNA contributes to bacterial adhesion to substrata, in addition to decreasing the environmental pH of bacterial biofilms, including in *P. aeruginosa* [136]. Increases in environmental pH are associated with decreased adhesion, and therefore the acidic environment created by eDNA within the biofilm is highly conducive to bacterial adhesion [134, 136]. Furthermore, acidic pH is associated with an increase in eDNA production and subsequent bacterial adhesion to substrata [137] as well as promoting the *P. aeruginosa* antibiotic resistance phenotype [136].

Khelissa et al. [138] demonstrated that both temperature and exposure time had a significant effect on the adhesion of *S. aureus* to both stainless steel and polycarbonate substrata, with an increase in either factor eliciting an increase in adherence. For example, an increase in the environmental growth temperature from 20 °C to 37 °C was associated with increased bacterial hydrophobicity, and thus the promotion of adhesion due to hydrophobic interactions in both *S. aureus* and *P. aeruginosa* [138, 139]. Proximity to the human core body temperature of 37 °C consistently facilitates bacterial adhesion to abiotic substrata; however, increases in temperature beyond this optimum are associated with a reduction in adhesion, hypothesized to be attributed to a decrease in bacterial viability. Pavlovsky et al. [140] demonstrated that heat treatment at 45 °C and 60 °C inhibited cell reproduction and viability respectively in *Staphylococcus epidermidis*, with the latter decreasing biofilm yield by an order of magnitude when compared with cultures at 37 °C. Prolonged exposure times coupled with an increase in temperature also affected acid-base interactions by decreasing the electron donor properties of *S. aureus*, thus attenuating the effect of the repulsive acid-base interaction between bacteria and the substratum and subsequently increasing adhesion [138]. The Derjaguin, Verwey, Landau, and Overbeek (DLVO) theory quantitatively describes the force between charged

surfaces interacting through a liquid medium and combines the effects of the van der Waals attraction and the electrostatic repulsion due to the so-called layer of counterions. However, due to the profound effect of Lewis acid-base interactions on *S. aureus* adhesion, its properties cannot be accurately predicted using this theory as is the case with *P. aeruginosa* [139]. Finally, the presence of antibiotics decreases bacterial adhesion according to bacterial susceptibility and antibiotic concentration [2, 141].

2.5.2 Biomaterial Surface Properties and Surface Modification

Once in contact with a material, the bacterium is able to engage in interactions dependent on the surface characteristics of both the bacterium and the material surface. The contemporary medical devices used clinically consist of a broad range of biomaterials including naturally derived, synthetic, semisynthetic, and composite materials composed of many forms of metals, ceramics, and polymers. Surface properties such as surface chemistry, roughness, surface energy, and surface charge are known to be the major factors that influence initial bacterial adhesion and biofilm formation on implant surfaces [142]. In order to improve their biocompatibility, surface modification of polymers via plasma-processing techniques usually produces numerous functional groups and chemical cross-links, and treatments often cause severe degradation of the surface, leading to increased roughness as well as to surface heterogeneity. However, other surface modification techniques, such as the application of a polymer coating [143] or metal ion-mediated coatings [144], have been used to modify the polymeric surface and showed significant bactericidal effect. Additionally, time-dependent conformational rearrangements of these surfaces may also be observed, where all will influence the bacterial response. Oh et al. [145] investigated the combined effects of substrate hydrophobicity and zeta potential on the dynamics and kinetics of the initial stages of bacterial pathogens *S. aureus* and *E. coli*, and found that bacterial adhesion was greatest on hydrophilic substrates with positive surface charge characteristics, followed by hydrophobic substrates with a negative surface charge, and the least adhesion on hydrophilic substrates with negative surface charge characteristics. A study by Katsikogianni et al. [146] investigated the response of *S. epidermidis* to materials with specific chemical functionalities under controlled flow conditions, and demonstrated that an increase in the material surface free energy significantly reduced the adhesion of this hydrophilic bacterial strain. Therefore, modification of surface chemistry via changing the surface charge and hydrophobicity significantly influences the outcomes of bacterial adhesion and aggregation [147].

Generally characterized as average surface roughness (R_a) and root-mean-square surface roughness (R_q), the influence of surface roughness on the host cellular response, especially in dental and orthopedic implants, has been

extensively investigated. It is generally considered that implant surface roughness modulates the immune cell response and regulates bone cell proliferation, differentiation, and extracellular matrix protein deposition, with a rougher surface promoting increased bone integration [148]. Therefore, surface roughness and the "race for the surface" represent another important influencer for bacterial adhesion and subsequent biofilm formation. To date, contradictory results involving the degree of surface roughness that either promotes or discourages bacterial adhesion have been reported by various scholars. For example, Lucas et al. investigated the surface roughness of polymethylmethacrylate and *S. sanguinis* adhesion and found that a reduction in surface roughness was directly related to a decrease in bacterial adhesion [149]. Additionally, Li et al. [150] also found that an increased surface roughness had a positive effect and promoted *Streptococcus* spp. adhesion when compared to smooth and polished surfaces. However, other researchers have found either none or the opposite correlation of surface roughness and bacterial adhesion [151].

In nature, biological organisms such as plants, insects, and marine animals have dynamically adapted to survive the harsh natural environment, by evolving various micro- and nano-textured surfaces, which offer self-cleaning, antifouling, and antibacterial properties [152]. Therefore, investigating the surface topography of natural surfaces such as leaves, wings, eyes, legs, and skins has enlightened scientists into developing some of the most fascinating biomedical implant surfaces that are able to regulate initial bacterial adhesion, and microbial accumulation and proliferation. A pioneering study by Ivanova et al. [153] investigated the nanostructure of cicada wings and the adhesion of *Pseudomonas aeruginosa* on the wing surface. This study identified the presence of nanopillar arrays over the wing surface and showed its bactericidal effect via direct penetration of the bacteria. In a subsequent study by Keheller et al. [154], the authors revealed that the bactericidal properties of cicada wings were strongly correlated with the scale of the nanotopography present on the surface. Encouraged by this discovery, attempts to mimic the nanoprotrusions found in nature have in recent years led to the development of novel synthetic materials such as titanium dioxide (TiO_2) nanopillars to control bacterial infection [155]. This study found that mimetic TiO_2 nanopillars induced deformation and penetration of the Gram-positive and Gram-negative envelope, and suggested that the nanopillar efficacy was mediated by oxidative stress and did not necessarily require bacterial lysis to induce cell death. Other nature-inspired surface modifications of polymeric biomaterials have also shown promising results against bacterial colonization. For example, a photocatalytic shark-skin-patterned polymeric surface with TiO_2 nanoparticles resulted in a significant reduction in *Escherichia coli* attachment and inactivation when compared with smooth counterparts [156]. Therefore, furthering our understanding of how biological organisms control bacterial adhesion will undoubtedly continue to inspire the development of novel biomaterials with anti-adhesion and bactericidal characteristics.

2.6 Biofilm Formation

A biofilm is a functional multilayered community of microorganisms, adhering to an abiotic or biotic surface and organized within a self-produced exopolymeric matrix. Of note, biofilms can also exist as non-surface aggregates. The formation of biofilm is the main pathogenetic mechanism leading to the chronicity and irreducibility of infections. Biofilms are complex mixtures of proteins, eDNA released by bacterial autolysis, lipids, and polysaccharides surrounding bacterial communities as protective barriers that are biochemically modified during the bacterial life cycle. Within biofilms, bacterial cells develop into organized and complex communities with structural and functional heterogeneity resembling multicellular organisms in which water channels serve as a rudimentary circulatory system. Additionally, release of cell-to-cell signaling molecules (quorum sensing) induces bacteria within a population to respond in concert by changing patterns of gene expression involved in biofilm differentiation [2, 4]. Cells near the liquid-biofilm interphase are metabolically very active due to the abundance of oxygen and nutrients while also being susceptible to antibiotics and other antibacterial therapeutics. However, cells within the biofilm barrier experience a diffusion gradient of nutrients and oxygen. For example, the diffusion rate of oxygen through a biofilm is only 60% of the diffusion rate through water, and oxygen and nutrients are also actively consumed while diffusing through the biofilm [157, 158]. Additionally, oxygen is consumed by the polymorphonuclear leukocytes that attack the biofilm [159]. A pH gradient is also established, and in *P. aeruginosa*, this gradient is reported to range from 5.5 at the center of the microcolony to 7 near the bulk fluid [160]. Therefore, in the deeper layers, bacteria experience starvation-induced dormancy and form persister cells, which are metabolically inactive and, as a result, can survive very high levels of antibiotic exposure. Furthermore, the diffusion-limited transport results in a gradient of antibiotic exposure to the biofilm resulting in suboptimal killing which has been demonstrated to enhance antibiotic tolerance through increased biofilm formation.

Research performed in many biofilm-forming organisms has revealed that the development of a biofilm is a two-step process involving an initial attachment (in which bacteria adhere to a surface) and a subsequent maturation phase (when a 3D structure evolves), both being physiologically different and requiring phase-specific factors. The life cycle of a typical biofilm contains an early planktonic phase, where cells irreversibly adhere to the biomaterial and/or tissue surface. The maturation phase requires intercellular aggregation, during which bacteria divide and accumulate [161]. The cells within mature biofilms produce compounds that can induce shift from biofilm back to a planktonic mode of life; thus, a final detachment (or dispersal) phase involves the detachment of single cells or cell clusters by various mechanisms and is believed to be crucial for the dissemination of the bacteria to new infection sites in the human body. Biofilm dispersion is promoted by dispersant factors, such as proteases, DNAses, and surfactant molecules, for example, PSM peptides released by *S. aureus* from within the biofilm contribute to bacterial cell dissemination and the settlement of new biofilms at a distant site [162]. Biofilm

formation can be altered via changes in environmental conditions, e.g., phosphate starvation, nitrogen starvation, increased NaCl concentration, dehydration, pH, temperature, bacterial motility, and host-derived factors, leading to production of exopolysaccharides and consolidated adhesion [163].

2.6.1   Staphylococcus aureus *Biofilm Formation*

Staphylococcus aureus and *Staphylococcus epidermidis* are the leading etiologic agents of implant-related infections. *S. aureus* strains have been shown to regulate the production of different types of matrices depending on environmental conditions, thus enabling them to readily switch between polysaccharide- and protein-based biofilms [164]. Sugar and protein surface structures of the bacterium play a significant role in their persistence at the infection site. These components act as key agents in cell viability, virulence, and evasion of host defenses. The EPS of staphylococcal biofilms also contains eDNA, proteins (released from lysed cells as well as host proteins), and amyloid fibrils [165]. The major polysaccharides of staphylococcal biofilms include capsular polysaccharide (CP), cell wall teichoic acid (WTA), and polysaccharide intercellular adhesin/poly-ß (1–6)-N-acetylglucosamine (PIA/PNAG) [166]. They each play distinct roles in colonization, pathogenesis, and the evasion of the host immune defenses, and each is being explored as targets for antimicrobial interventions and therapeutics.

2.6.1.1   Capsular Polysaccharides

Polysaccharide capsules are structures found on the cell surface of a broad range of bacterial species. The EPS is often involved in mediating direct interactions between bacteria and its environment and therefore plays a key role in the important mechanisms of bacterial pathogenicity, most notably biofilm formation and immune evasion. The EPS may be classified either as CPs, where the polysaccharide is intimately associated with the cell surface, or as slime polysaccharides, where the polysaccharide is loosely associated with the cell. Differentiation between these forms is often difficult. CPs are highly hydrated molecules and can be either homo- or heteropolymers composed of repeating monosaccharides joined by glycosidic linkages [167]. They are considered to function in the prevention of desiccation, adherence, resistance to nonspecific host immunity, and resistance to specific host immunity and in mediating the diffusion of molecules through the cell surface. Bacteria causing invasive diseases produce CPs that serve as essential virulence factors, and in the case of human pathogens, a large number of different capsule serotypes have been identified, and certain CPs or K antigens have been associated with specific infections [168]. CPs are produced by the majority of clinical isolates of *S. aureus.* For example, the *S. aureus* CPs (CP5 and CP8) have been shown to possess antiphagocytic properties, allowing the bacterium to persist in the blood and tissues of infected

hosts [91]. Production of CPs on the bacterial surface effectively masks the pathogen and its surface-associated proteins, such as opsonins, from recognition by phagocytic cells. Additionally, secretion of the extracellular fibrinogen-binding protein (Efb) potently blocks phagocytic uptake of the pathogen. Efb creates a fibrinogen shield surrounding the bacteria by simultaneously binding complement C3b and fibrinogen at the bacterial surface. CP5 O-acetylation rendered *S. aureus* more resistant to opsonophagocytic killing by human neutrophils [169]. Purified CP8 was shown to activate CD4+ T cells in vitro, and purified CP5 and CP8 facilitated intra-abdominal abscess formation when administered to rats with an adjuvant [170]. As described above, host TLRs are critical in innate and adaptive immune responses because they sense invading pathogens and signal the production of proinflammatory cytokine responses and prime Th1 and Th17 responses. CPs mask TLR2 activity in *S. aureus* by interfering with lipoprotein recognition by TLR2. Due to their activity, CP5 and CP8 conjugate vaccines are believed to be important components for a multivalent staphylococcal vaccine, and there are many ongoing trials that include multicomponent vaccines that have reached early clinical trials [171].

2.6.1.2   Polysaccharide Intercellular Adhesin/Poly-N-Acetylglucosamine

Following attachment to a surface, bacteria multiply and form multicellular aggregates as the cells begin to adhere to each other. Staphylococci mediate cell-to-cell adhesion using two types of exopolymers: PIA and surface proteins. During biofilm formation, the cationic PIA within the EPS forms an extracellular matrix that connects the cells together within a fibrous net, building up biofilm mass and its resistance to mechanical force [172] (Fig. 2.4). The exposed positively charged NH^{3+} groups of PIA, introduced by the deacetylation of N-acetylglucosamine residues, are essential for this to happen, as it allows for the molecules within the matrix to attach to the negatively charged bacterial cell surface via electrostatic interactions [173, 174]. As such, PIA is crucial for biofilm formation. PIA/PNAG is now recognized as a highly conserved polysaccharide antigen produced by many microbes and is known to play a key role in adherence to a biomaterial surface [164]. PIA is a PNAG that is partially deacetylated and positively charged, and whose synthesis is mediated by the *icaADBC* locus. The *icaADBC* genes play an important role in biofilm formation where the N-acetylglucosaminyltransferase enzyme is encoded by the *icaA* gene and is responsible for PIA production, while *icaD* is involved in enhancing the activity of the enzyme for complete phenotypic expression of PIA [175]. The EPS is also partially (15–20%) deacylated by the IcaD protein [176]. Poly-*N*-acetyl-D-glucosamine (GlcNAc) is a core polysaccharide unit with connected by β1–6-glycosidic linkages that is not found in mammals; however, it is expressed by many microbes, including both Gram-negative and Gram-positive bacteria, fungi, and protozoan parasites [177]. *As mentioned, bacterial surface protein activity is also critical for biofilm development alongside PIA.* Surface proteins, such as biofilm-associated protein, surface protein G (SasG), extracellular adherence protein, and fibronectin-binding proteins, can contribute to intercellular
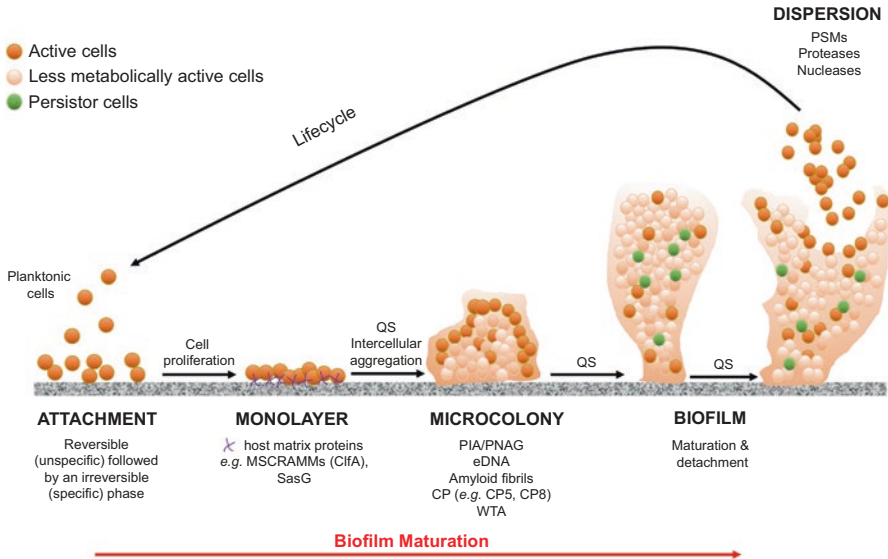
Fig. 2.4 Stages of *S. aureus* biofilm formation, maturation, and dispersion. Attachment of cells to a surface occurs via hydrophobic interactions to an abiotic surface, or via surface proteins that bind in a specific fashion to host matrix proteins covering an implanted medical device. Growth of the biofilm in the proliferation/maturation stage is accompanied by the production of cell-cell-adhesive matrix components (such as PIA, eDNA, and proteins) as well as disruptive factors (such as PSMs and degradative secreted enzymes). Those disruptive factors can also cause detachment, with the release of PSMs, proteases, and nucleases which aid dispersion

adhesion. *S. aureus* SasG is closely related to the accumulation-associated protein (Aap), which is needed for biofilm formation. The G_5-E domains of SasG become exposed by proteolytic removal of the N-terminal A domain of Aap or by the limited cleavage within the G_5-E domain of SasG [166, 178]. This allows a specific homophilic interaction to occur between proteins located on adjacent cells, which promotes cell aggregation and biofilm accumulation [179].

2.6.1.3 Wall Teichoic Acid

One of the major differences between Gram-negative and Gram-positive organisms is the presence or absence of an outer membrane. Gram-negative bacteria contain an outer membrane that protects the organism by filtering out toxic molecules while also serving as a scaffold to which proteins and polysaccharides that mediate interaction between the pathogen and its environment are anchored. In contrast, Gram-positive bacteria lack an outer membrane but instead consists of thick layers that envelope the bacteria within a complex cell casing. This casing varies in structure, but all contain layers of peptidoglycan (PG), a cross-linked matrix of linear carbohydrate (glycan) chains linked to one another via covalent bonds between attached

peptides [180]. This PG matrix is essential for survival, and the PG layers of many Gram-positive bacteria are densely functionalized with anionic glycopolymers called WTA, a ribitol-phosphate surface polymer modified with GlcNAc. Teichoic acids (TAs) are the most abundant of PG-linked polymers and are produced by all strains of *S. aureus*; however, WTA structures are highly diverse among Gram-positive bacteria and are often strain- or species-specific. There are two types of TAs: the lipo-TAs, which are anchored to the plasma membrane and extend from the cell surface into the PG layer, and the WTAs, which are covalently attached to PG and extend through and beyond the cell wall. Together they create a "continuum of negative charge" on the cell surface. Owing to their location, abundance, and poly-anionic nature, WTA plays numerous and varied roles, including cell wall mainte-nance and shape, cell division, PG synthesis, ion homeostasis, autolysis, colonization, and resistance to antimicrobial agents [181]. Additionally, WTA and its attached substituents contribute to bacterial cell surface-charge and hydrophobicity, which affects the binding of extracellular molecules, thus playing a role in protecting bac-teria from adverse threats, such as from antimicrobial surfactants to bacteriolytic enzymes [182]. Wall teichoic acids are polymers of sugars and alcohol phosphates and, which similar to EPS, have been implicated in colonization and biofilm forma-tion. However, due to their ubiquitous presence and covalent surface linkage, they are generally not considered EPS. Wall teichoic acids are not required for the growth of *S. aureus* in vitro; however, the presence of their D-alanyl esters mediates bacte-rial interaction to tissue and biomaterial surfaces, as it has been shown that bacteria deficient in WTAs demonstrate a reduced ability to form biofilms [183]. Moreover, several animal studies have established that WTA-deficient bacteria attenuated host colonization and infection [180, 184]. D-alanyl esters are considered virulence fac-tors, since their deletion attenuates pathogenicity but does not cause major cellular defects and their overexpression increases *S. aureus* virulence [185]. It has also been suggested that WTAs can act as an "immunological cloak" for *S. aureus*, pre-venting antibodies from recognizing and opsonizing the cell wall [181]. This may be of high importance for the cell as PG is absent in mammalian tissues, however, is found in high abundance on the bacterial surface, and so represents an ideal target for the immune system. A recent study by Gautam et al. reported a previously unrecognized immunoevasive role for WTAs in that they contributed to the repul-sion of PG-targeted antibodies. Human immune receptors from multiple classes recognize glycan modifications on *S. aureus* WTAs and have been shown to have important immunostimulatory activities, and a large percentage of human antibod-ies against S. aureus is directed against WTA [182]. It is currently unknown whether the immune evasion capacities of MRSA are due to variation of dominant surface epitopes such as those associated with WTA. For example, D-ala residues on WTA (and LTA) contribute to resistance to cationic antimicrobial peptides such as defen-sins or cathelicidins as well as to glycopeptide antibiotics such as vancomycin and teicoplanin. Due to their importance in pathogenesis, WTAs are targets for new therapeutics to overcome resistant bacterial infections. Indeed, the first WTA-active antibiotic has been reported.

2.6.1.4 Quorum Sensing

Bacterial to bacterial communication relies on versatile chemical signaling oligo-peptides called autoinducers, which regulate bacterial gene expression in a process known as quorum sensing. Quorum sensing allows groups of bacteria to synchronously alter behavior in response to changes in the population density and species composition. Coordinated behaviors include bioluminescence, virulence factor production, secondary metabolite production, competence for DNA uptake, and biofilm formation [186]. These processes are futile when undertaken by a single bacterium acting alone. Quorum-sensing-mediated communication is now understood to be the norm in the bacterial world, and like languages between humans, these signals vary between species. During their reproductive cycle, individual bacterium synthesizes autoinducers. Gram-positive autoinducers are made of peptide and must be actively transported through the PG cell wall using the ATP-binding cassette (ABC) transporter system. Autoinducers move out of individual cells as they are produced, and as cell density increases, the autoinducer concentration rises in the extracellular environment until a threshold intracellular concentration is exceeded. At this "critical mass" concentration, the threshold produced makes it energetically unfavorable for the intracellular autoinducers to leave the cell, resulting in the autoinducer binding to their receptors, and this complex then acts to induce or repress the expression of target genes. This cell density-dependent regulation of virulence factor production has been suggested as a protective means to prevent the host response to invading bacteria before sufficient bacterial numbers have accumulated.

Quorum sensing is essential to ensure the progress of the three stages of biofilm formation. The *S. aureus* quorum-sensing system involves two regulatory systems, the accessory gene regulator (Agr) system and the LuxS system. The Agr locus consists of two divergent transcripts, RNAII and RNAIII, initiated from two distinct promoters, P2 and P3, respectively, and produces a communication molecule called autoinducing peptide (AIP) [187]. Once AIP reaches a critical concentration, a regulatory cascade is initiated, and a myriad of virulence factors are expressed. As such, the upregulation of virulence factors by Agr is necessary for disease progression, with α-toxin being one of the most prominent. The P2 operon contains agrBDCA and codes for the RNAII transcript, while P3 drives transcription of the effector molecule of the agr locus (RNAIII). An increase in the transcription of P2 and P3 appears to result in a rise of the intracellular concentrations of RNAIII, which, in turn, also increases the expression of secreted virulence factors such as α-hemolysin. Conversely, downregulation by Agr of PSMs and microbial surface components has been implicated in enhanced biofilm formation and bacterial colonization of indwelling medical devices. Moreover, Agr dysfunction is correlated with persistent *S. aureus* bacteremia [188]. At the beginning of an infection, low cell density and subsequently the low expression of Agr result in an increase in the production of the surface proteins required for the initial colonization of tissues. Once this is established, bacteria grow to higher cell densities, requiring additional food sources and increased protection from host defenses, which is accomplished by Agr-dependent

upregulation of degradative exoenzymes, leukocidins, and exotoxins [188]. Of interest, subinhibitory concentrations of antibiotics have been shown to increase Agr expression imposing an energy-consuming cost, which has been speculated to drive the observed formation of Agr-dysfunctional mutants in strains isolated from hospital infections. The impact of Agr on biofilm-associated infection is divergent: Agr is necessary for biofilm structuring and the dissemination of biofilm infection, but dysfunction of Agr leads to enhanced biofilm formation, which may be advantageous for the bacteria under those conditions. Accordingly, strains with a dysfunctional Agr system are often isolated from infections on implanted devices [189]. Recently salicylic acid has been shown to impact the quorum-sensing system via the *agr* locus, limiting the bacterial cell escape from within the biofilm while maintaining high biomass in the mature biofilm, which can promote tolerance to antibiotics and make the infection refractory to the recommended antibiotic therapy [162].

The regulatory effect of *luxS* was discovered in the context of bioluminescence regulation, and since has been recognized as a widely utilized quorum-sensing system among bacteria. The LuxS system employs an autoinducer called AI-2, which is a furanosyl borate diester molecule. Several phenotypes, such as capsule synthesis, biofilm formation, antibiotic susceptibility, and virulence, have been linked to AI-2 regulation in *S. aureus* [188, 190, 191].

2.6.2   Pseudomonas aeruginosa *Biofilm Formation*

Due to their clinical importance, *P. aeruginosa* biofilms are one of the most studied single-species biofilms, and over the years, certain themes regarding *P. aeruginosa* biofilms have emerged and been considered dogmatic. *P. aeruginosa* attaches to catheters, drains, implants, or lenses, for example, causing serious infections despite rigorous cleaning and disinfection procedures [192]. As such, colonization of *P. aeruginosa* in device-related and tissue infections in patients, along with the emergence of alginate-producing mucoid variants, is considered a poor prognostic indicator, as these infections can be highly challenging to eradicate using current therapeutic strategies [193].

P. aeruginosa swims rapidly in liquid by means of flagella, and during biofilm formation this motility is involved in initial location and adherence to solid surfaces [194]. Biofilm development progresses in five stages: (i) reversible attachment, in which motile (planktonic) cells attach to a surface using the flagellum; (ii) irreversible attachment, where cells become more firmly connected to the surface via the long axis of the cell; (iii) microcolony formation, in which cells aggregate and secrete matrix components; (iv) mature biofilm, characterized by macrocolony and fluid channel formation; and (v) dispersal. Prior to *P. aeruginosa* adhering to a surface, the organism can be seen to swim along the surface, almost as if it is scanning for an appropriate location for initial contact. Once attached, prokaryotes have the ability to walk on surfaces using a flagellum-independent motility known as twitching, and powered by the extension and retraction of type IV pili. When twitching,

P. aeruginosa moves linearly along their axis and is motile only in large groups (swarming), suggesting this is a community behavior. Pili retraction motors can generate up to 100pN; however, how such large forces power cell body displacements or how any surface motile organism is able to direct movement is poorly understood [195]. Wild-type, non-mucoid *P. aeruginosa* biofilm formation progresses via distinct steps. Following the adhesion of single cells to the surface and cell twitching to form clumps or microcolonies, they continue to proliferate to form a mature biofilm consisting of several layers of stacked cells. The cells account for 10% of the biomass and produce EPS, which makes up 90% of the biofilm. eDNA is another important structural component for *P. aeruginosa* biofilm development through providing multifaceted roles such as contributing to forming cation gradients in the matrix via the chelating interaction of highly anionic DNA with cations such as Mg^{2+}, Ca^{2+}, Mn^{2+}, and Zn^{2+} [196], as a nutrient source during starvation, in facilitating twitching motility, coordinating cell movements, and conferring antibiotic resistance [197].

P. aeruginosa is capable of producing multiple EPSs, including Psl, Pel, and alginate, which are considered to be involved in the surface attachment, formation, and the stability of *P. aeruginosa* biofilm architecture. These polysaccharides differ in chemical structure and in their biosynthetic mechanisms. Pel is an N-acetylglucosamine and N-acetyl galactosamine-rich polysaccharide that is cationic under slightly acidic pH and interacts with eDNA in the matrix. Psl is composed of a neutral pentasaccharide subunit containing mannose, rhamnose, and glucose, and alginate is a negatively charged acetylated polymer of guluronic and mannuronic acid. The ability to produce three EPSs, each with a different charge at physiological pH, may afford *P. aeruginosa* biofilms increased flexibility to maintain biofilm structure and/or protect cells from antimicrobials under different conditions [198]. The amount of these polysaccharides within biofilm varies across *P. aeruginosa* strains, with Pel and Psl predominating in the non-mucoid phenotype and where alginate overproduction is characterized by the mucoid phenotype [199]. Since Psl and Pel were discovered fairly recently, the biosynthetic mechanisms of these two EPSs are not well established, and many aspects of alginate biosynthesis still remain unclear. The Psl EPS is necessary for the initial steps of biofilm formation in both non-mucoid and mucoid strains. It is anchored around cells in a helical arrangement such that it enhances cell migration, cell-cell interaction, and cell-surface adhesion. In mature biofilms it is located to the periphery of mushroom-shaped microcolonies [74]. Psl provides an immediate protective role against anti-biofilm agents and a broad spectrum of antibiotics particularly during the early stage of biofilm development [200]. Therefore, Psl provides a survival advantage during pathogenesis. Presently, much remains unknown regarding Pel's spatial organization and role in microcolony formation. Pel has been shown to promote biofilm tolerance to aminoglycoside antibiotics [201], while the positive charge imparts important functional characteristics such as cross-linking eDNA within the biofilm stalk region via ionic interactions [198]. It is possible that the cross-linking of eDNA to other cationic exopolysaccharides (e.g., PNAG) may be a general mechanism important for the structural integrity of biofilms, while the cross-linking

and binding of Pel to host polymers such as hyaluronan and mucin, both abundant at sites of infection, have implications in terms of enhancing disease pathogenesis. However, the importance of Pel/eDNA interaction is currently unknown and an active area of research. The important functions of alginate include biofilm maturation, protection from phagocytosis and opsonization, and decreasing the diffusion of antibiotics through the biofilm matrix [202]. Additionally, alginate can greatly influence biofilm characteristics such as its viscoelastic properties, bio-volume, cell density, and architecture as well as cell-to-cell interaction, cell aggregation, and surface attachment [203].

Less is known about the identity and function of *P. aeruginosa* biofilm matrix proteins. The most studied is the extracellular adhesin CdrA, which promotes aggregate formation through Psl interactions when under planktonic conditions, and helps stabilize and maintain biofilm structural integrity. It has been shown to promote bacterial aggregation in the absence EPS [204, 205]. No other matrix proteins that play a role in the structural stability of *P. aeruginosa* biofilm have been found. The protein ecotin is reported to contribute to bacterial defense against neutrophil elastase [206] and the Fap amyloid proteins, in biofilm stiffness [207]. *P. aeruginosa* produces two small soluble lectins, galactophilic lectin (LecA) and LecB (also named PAI-L and PAII-L, respectively) that bind galactose and fucose, respectively, as well as oligo- and polysaccharides containing these sugars. Their primary functional role is to mediate attachment to the host during infection. For example, LecA is involved in host cell invasion and cytotoxicity, while LecB reduces ciliary beating of airway epithelium [208, 209]. LecA induces an increased permeability of intestinal and respiratory epithelial cells enabling cytotoxic exoproducts such as ExoA to enter host cells [210]. The lectins are mostly localized within the cell cytoplasm, and both are also linked to biofilm formation on abiotic surfaces, although the underlying mechanisms are presently unknown. More recently, multiple proteomic analyses of the soluble *P. aeruginosa* biofilm matrix identified the outer membrane (OM) porin OprF, as an abundant matrix protein [211]. Porins are integral OM proteins that form hydrophilic channels through which charged solutes can pass; however, the role of OprF has been relatively understudied. Song et al. recently reported that OprF is necessary for *P. aeruginosa* to sense surface stiffness during the attachment stage of biofilm formation [212]. Additionally, and during the immune response to *P. aeruginosa* infection, OprF is bound by C3b of the complement system, which tags the bacteria for phagocytosis by host macrophages and neutrophils. Interferon-γ (IFN-γ) produced by T cells was shown to bind directly to *P. aeruginosa* OprF, and upon formation of IFN-γ-OprF complexes, the *rhl* QS system was activated and resulted in upregulation of the expression of *lecA* and the synthesis of the toxin pyocyanin [213]. The production of Pel polysaccharide and eDNA and QS-controlled production of pyocyanin are critical for biofilm maturation. Furthermore, pyocyanin molecules can promote eDNA release by inducing bacterial cell lysis. Pyocyanin binds to eDNA increasing its solution viscosity which influences the physicochemical interactions of the biofilm matrix with environment as well as facilitates cellular aggregations [214]. Collectively, such molecular and cellular interactions in combination with other polymeric substances lead to the establishment of a robust and mature biofilm.

2.6.2.1   Bacterial Detachment and Dispersion

Bacterial biofilm dispersal can be divided into three distinct phases: (i) detachment of cells from the biofilm colony; (ii) translocation of the cells to a new location; and (iii) attachment of the cells to a substrate in the new location. Bacterial detachment and dispersion, or effectively their escape from the biofilm, is a complex process that involves numerous environmental signals, signal transduction pathways, and effectors [215]. The environmental signals that may induce detachment of cells are nutrient starvation, oxygen tension, temperature, osmolarity, and pH, as well as proteins, including integration host factors. Additionally, interspecific AMPs, quorum-sensing signals, or matrix-degrading enzymes are examples of effectors involved in this process [216]. Dispersed cells move along the solid surface via a convection current before reattaching to the surface at a new location, where they form a new biofilm colony. This can result in the appearance of streamers of satellite colonies emanating from the dispersed biofilm colony. Mechanisms of biofilm dispersion can basically be divided into two groups, active and passive dispersion. Active dispersion depends on a decrease in the intracellular cyclic diguanylate (c-di-GMP) levels, leading to the production of enzymes that degrade the biofilm matrix and promote dispersion. This response is normally triggered by an environmental change through the activation of phosphodiesterases (PDEs), which decrease the c-di-GMP level, resulting in the production of matrix-degrading enzymes, causing dispersal [158]. Interestingly, low intracellular c-di-GMP concentrations promote the planktonic lifestyle, while high concentrations stimulate life as a biofilm [217]. In contrast, passive dispersion results from either physical disruption or through enzymatic degradation, which relies on triggers that are directly released from cells. Both mechanisms can lead to the release of single cells or clumps of biofilm [218]. Passive dispersion occurs independently of c-di-GMP concentration, and the physical detachment of biofilm from a surface occurs via four mechanisms: (i) abrasion (collision of solid particles with the biofilm); (ii) grazing, which involves the removal of cells by active eukaryotic predators; (iii) erosion due to fluid shear; and (iv) the sloughing off of larger pieces of biofilm by fluid shear [219]. Seeding dispersal, also known as central hollowing, refers to the rapid release of a large number of single cells or small clusters of cells from hollow cavities that form inside the biofilm colony [220].

During both active and passive dispersions, biofilm cells produce matrix-degrading enzymes, such as glycosidases, proteases, and deoxyribonucleases [209]. In particular, the biofilm matrix-degrading enzyme dispersin B, a ß-hexosaminidase, has proven effective in many Gram-negative pathogens, via hydrolyzing the (ß-1,6)-glycosidic linkages of PNAG, resulting in an 85% reduction in biofilm mass when administered in vitro [221]. Although mainly studied in *A. actinomycetemcomitans*, homologous genes are present in the genomes of several other bacteria. The *P. aeruginosa* glycoside hydrolase PelA is produced when dispersion is induced, and the exogenous administration of PelA and PslG induces biofilm dispersion and prevents biofilm formation [222]. In mucoid strains of *P. aeruginosa*, alginate lyase degrades endogenous matrix components and mediates biofilm cell detachment, while its

overexpression has been reported to accelerate detachment and cell sloughing from biofilms [223].

A potential approach to combat biofilm-related infections is to induce biofilm dispersion and thereby enhancing the susceptibility of cells to antibiotics, for example. Lowering the concentration of c-di-GMP by itself does not necessarily result in biofilm dispersion, nor through upregulation of PDEs [158, 224]. One of the first molecules identified as a *P. aeruginosa* biofilm-dispersing agent was nitric oxide, and although toxic, it can induce dispersion at low concentrations, leaving the cells susceptible to antibiotic treatment [225]. Heavy metals such as iron, mercury chloride, silver nitrate, and sodium arsenate disperse *P. aeruginosa* biofilms. While the use of mercury chloride and sodium arsenate is questionable due to their high toxicity, silver nitrate and silver nanoparticles are areas of current active research. In addition to a sudden increase of nutrients, nutrient depletion also induces biofilm dispersion in vitro, with a 60% reduction in *P. aeruginosa* biofilm biomass reported after 24 h of glucose depletion [158, 226]. As described above, dispersin B is highly effective at causing biofilm dispersion, and studies have shown that combining dispersin B with cefamandole nafate or triclosan improved biofilm eradication (*S. aureus* and *S. epidermidis*) when compared with either antibiotic alone [227, 228]. A combined treatment of dispersin B with tobramycin reduced the number of bacteria in a *S. aureus* biofilm by 7500-fold in comparison with tobramycin alone, which reduced the cell number by only 40-fold [229]. Additionally, and in vivo, a DispersinB-based wound spray was able to eradicate a MRSA biofilm by 80% when compared with a silver wound dressing (14%) [230].

2.6.2.2   Quorum Sensing

Similar to *S. aureus*, *P. aeruginosa* enters into the QS mode in response to changes in cell density or due to environmental cues or stresses and involves the production, secretion, and accumulation of autoinducers. As described for *S. aureus*, once a critical concentration of autoinducer is achieved, it binds to the regulatory protein, and this complex acts to induce or repress the expression of target genes. Also, and as with *S. aureus*, QS has been linked to the regulation of virulence factor production, stress tolerance, metabolic adjustment, and host-microbe interactions [231]. In brief, there are four distinct *P. aeruginosa* QS pathways, namely, Las, Rhl, PQS, and IQS. Each produces their respective intracellular cognate autoinducers, i.e., *N*-3-oxo-dodecanoyl-L-homoserine lactone (3O-C12-HSL), *N*-butyryl-L-homoserine lactone (C4-HSL), 2-heptyl-3-hydroxy-4-quinolone (HHQ), and 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde (IQS), respectively. These systems function interdependently, and all have been shown to be important in its pathogenesis as well as in the production of the fundamental elements involved in biofilm formation [232]. For example, 3O-C12-HSL and C4-HSL bind to and activate their cognate transcription factors LasR and RhlR, respectively, inducing biofilm formation and expression of various virulence factors including elastase, proteases, pyocyanin, lectins, rhamnolipids, and toxins [233]. The QPS system is able to regulate
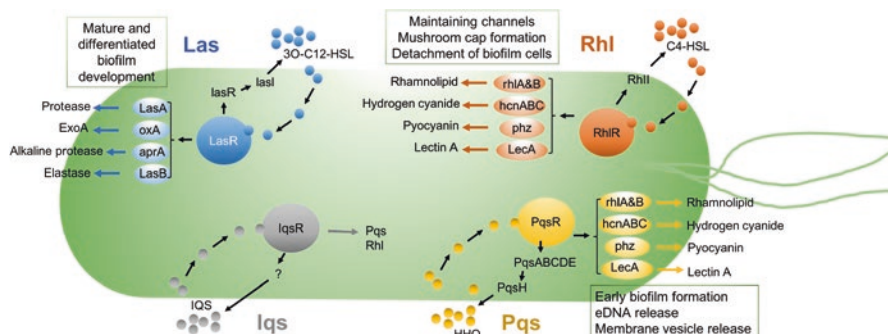
Fig. 2.5 The four QS systems of *P. aeruginosa*, Las, Rhl, Pqs, and Iqs, produce the autoinducers 3O-C12HSL, C4-HSL, HHQ, and IQS, respectively. Examples of the virulence factors produced by each system are shown and the role of each system in biofilm maturation. Much debate remains as to the mechanism and role of the IQS system and the contribution if any, of ambABCDE in its regulation [236]

biofilm formation and induce exogenous dispersal through the proteins PqsA and PqsD [234]. Finally, the QS-dependent production of rhamnolipids has a crucial role in neutralizing the attack of neutrophils due to their necrotic property [235]. This description is by no means exhaustive, but only briefly summarizes the highly complex OS system of *P. aeruginosa* (Fig. 2.5).

References

1. Trampuz A, Osmon DR, Hanssen AD, Steckelberg JM, Patel R. Molecular and antibiofilm approaches to prosthetic joint infection. Clin Orthop Relat Res. 2003*; (414):69–88.*
2. Ribeiro M, Monteiro FJ, Ferraz MP. Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. Biomatter 2012;2(4):176–194. https://doi.org/10.4161/biom.22905
3. Hoiby N, Ciofu O, Johansen ZJ, Song C, Moser PO, Jensen et al. The clinical impact of bacterial biofilms. Int J Oral Sci. 3(2) (2011):55–65.
4. Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. *Eur Cell Mater. 2004 Dec 7; 8():37–57.*
5. Turner IG, Pilliar RM, Srichana T, Domb AJ, Lacroix D, Planell JA, et al. Sterility and Infection. In: Narayan R, ed. Biomedical Materials. New York, NY: Springer Science, 2009:239–258.
6. Menkin, V. Studies on inflammation: VII. Fixation of bacteria and of particulate matter at the site of inflammation. *J. Exp. Med.* 53, 647–660 (1931).
7. Gristina, A. G. Implant failure and the immuno-incompetent fibroinflammatory zone. Clin. Orthop. Relat. Res. 298, 106–118 (1994).
8. Schierholz, J. M. & Beuth, J. Implant infections: a haven for opportunistic bacteria. J. Hosp. Infect. 49, 87–93 (2001).
9. Christo, S. N., Diener, K. R., Bachhchuka, A., Vasilev, K. & Hayball, J. D. 2015. Innate Immunity and Biomaterials at the Nexus: Friends or Foes. Biomed Res Int*, 2015, 342304.

10. C. J. Nonckreman, S. Fleith, P. G. Rouxhet, and C. C. Dupont-Gillain, "Competitive adsorption of fibrinogen and albumin and blood platelet adhesion on surfaces modified with nanoparticles and/or PEO," Colloids and Surfaces B: Biointerfaces, vol. 77, no. 2, pp. 139–149, 2010.

11. C. J. Wilson, R. E. Clegg, D. I. Leavesley, and M. J. Pearcy, "Mediation of biomaterial-cell interactions by adsorbed proteins: a review," Tissue Engineering, vol. 11, no. 1–2, pp. 1–18, 2005.

12. Marongiu L, Gornati L, Artuso I, Zanoni I, Granucci F. 2019. Below the surface: the inner lives of TLR4 and TLR9. J Leukoc Biol. 106:147–160.

13. Szaba, F. M. & Smiley, S. T. 2002. Roles for thrombin and fibrin(ogen) in cytokine/chemokine production and macrophage adhesion in vivo. Blood, 99, 1053–9.

14. Xu, L. C. & Siedlecki, C. A. 2007. Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces. Biomaterials, 28, 3273–83.

15. M. Ghasemzadeh, Z. S. Kaplan, I. Alwis et al., "The CXCR1/2 ligand NAP-2 promotes directed intravascular leukocyte migration through platelet thrombi," Blood, vol. 121, no. 22, pp. 4555–4566, 2013.

16. G. Nimeri, L. Öhman, H. Elwing, J. Wetterö, and T. Bengtsson, "The influence of plasma proteins and platelets on oxygen radical production and F-actin distribution in neutrophils adhering to polymer surfaces," Biomaterials, vol. 23, no. 8, pp. 1785–1795, 2002.

17. L. Liu, H. Elwing, A. Karlsson, G. Nimeri, and C. Dahlgren, "Surface-related triggering of the neutrophil respiratory burst. Characterization of the response induced by IgG adsorbed to hydrophilic and hydrophobic glass surfaces," Clinical and Experimental Immunology, vol. 109, no. 1, pp. 204–210, 1997.

18. E.-C. Shen, T.-C. Chou, C.-H. Gau, H.-P. Tu, Y.-T. Chen, and E. Fu, "Releasing growth factors from activated human platelets after chitosan stimulation: a possible bio-material for platelet-rich plasma preparation," Clinical Oral Implants Research, vol. 17, no. 5, pp. 572–578, 2006.

19. S. Chen, J. A. Jones, Y. Xu, H.-Y. Low, J. M. Anderson, and K. W. Leong, "Characterization of topographical effects on macrophage behavior in a foreign body response model," Biomaterials, vol. 31, no. 13, pp. 3479–3491, 2010.

20. K. Garg, S. A. Sell, P. Madurantakam, and G. L. Bowlin, "Angiogenic potential of human macrophages on electrospun bioresorbable vascular grafts," Biomedical Materials, vol. 4, no. 3, Article ID 031001, 2009.

21. T. Oviedo-Socarrás, A. C. Vasconcelos, I. X. Barbosa, N. B. Pereira, P. P. Campos, and S. P. Andrade, "Diabetes alters inflammation, angiogenesis, and fibrogenesis in intraperitoneal implants in rats," Microvascular Research, vol. 93, pp. 23–29, 2014.

22. J. Coia, G. Duckworth, D. Edwards, M. Farrington, C. Fry, H. Humphreys, C. Mallaghan, D. Tucker, J.W.P.o.t.B.S.o.A. Chemotherapy, Guidelines for the control and prevention of meticillin-resistant Staphylococcus aureus (MRSA) in healthcare facilities, Journal of hospital infection, 63 (2006) S1–S44.

23. Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, Lessa FC, Lynfield R, Nadle J, Petit S, Ray SM, Schaffner W, Townes J, Fridkin S, Emerging Infections Program–Active Bacterial Core Surveillance MRSA Surveillance Investigators. National burden of invasive methicillin-resistant Staphylococcus aureus infections, United States, 2011. JAMA Intern Med. 2013 Nov 25; 173(21):1970–8.

24. Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat Rev Microbiol. 2009 Sep; 7(9):629–41.

25. K.E. Bachta, J.P. Allen, B.H. Cheung, C.-H. Chiu, A.R. Hauser, Systemic infection facilitates transmission of Pseudomonas aeruginosa in mice, Nature communications, 11 (2020) 1–13.

26. N. Spernovasilis, M. Psichogiou, G. Poulakou, Skin manifestations of Pseudomonas aeruginosa infections, Current Opinion in Infectious Diseases, 34 (2021) 72–79.

27. O. Ciofu, T. Tolker-Nielsen, Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents—how P. aeruginosa can escape antibiotics, Frontiers in microbiology, 10 (2019) 913.

28. R. Roy, M. Tiwari, G. Donelli, V. Tiwari, Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action, Virulence, 9 (2018) 522–554.

29. Z. Pang, R. Raudonis, B.R. Glick, T.-J. Lin, Z. Cheng, Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies, Biotechnology advances, 37 (2019) 177–192.

30. D. Dey, L.G. Kavanaugh, G.L. Conn, Antibiotic substrate selectivity of Pseudomonas aeruginosa MexY and MexB efflux systems is determined by a Goldilocks affinity, Antimicrobial Agents and Chemotherapy, 64 (2020).

31. Muthukrishnan G, Masters EA, Daiss JL, Schwarz EM. Mechanisms of Immune Evasion and Bone Tissue Colonization That Make Staphylococcus aureus the Primary Pathogen in Osteomyelitis. Curr Osteoporos Rep. 2019;17(6):395–404. https://doi.org/10.1007/s11914-019-00548-4

32. Brinkman V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. 2004. Neutrophil extracellular traps kill bacteria. Science. 303:1532–1535.

33. Boeltz S, Amini P, Anders HJ, Andrade F, Bilyy R, Chatfield S, Cichon I, Clancy DM, Dessai J, Dumych T, et al. 2019. To NET or not to NET: current opinions and state of the science regarding the formation of neutrophil extracellular traps. Cell Death Differ. 26:395–408.

34. Bryzek D, Ciaston I, Dobosz E, Gasiorek A, Makarska A, Sarna N, Eick S, Puklo M, Lech M, Potempa B, et al. 2019. Triggering NETosis via protease-activated receptor (PAR)-2 signaling as a mechanism of hijacking neutrophils function for pathogen benefits. PLoS Pathog. 15:e1007773.

35. Kang J, Dietz MJ, Li B. 2019. Antimicrobial peptide LL-37 is bactericidal against *Staphylococcus aureus* biofilms. PLoS One. 14:e0216676. https://doi.org/10.1371/journal.pone.0216676

36. Shahrour H, Ferrer-Espada R, Dandache I, Bárcena-Varela S, Sánchez-Gómez S, Chokr A, Martínez-de-Tejada G. 2019. AMPs as anti-biofilm agents for human therapy and prophylaxis. Adv Exp Med Biol. 1117:257–279. https://doi.org/10.1007/978-981-13-3588-4

37. Koppen BC, Mulder PPG, de Boer L, Riool M, Drijfhout JW, Zaat S. 2019. Synergistic microbicidal effect of cationic antimicrobial peptides and teicoplanin against planktonic and biofilm-encased *Staphylococcus aureus*. Int J Antimicrob Agents. 53:143–151. https://doi.org/10.1016/j.ijantimicag.2018.10.002

38. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010 May; 11(5):373–84.

39. Campoccia D, Mirzaei R, Montanaro L, Arciola CR. Hijacking of immune defences by biofilms: a multifront strategy. Biofouling J Bioadh Biofl Res. 35 (10) (2019):1055–1074.

40. Bayer AS, Speert DP, Park S, Tu J, Witt M, Nast CC, Norman DC. Functional role of mucoid exopolysaccharide (alginate) in antibiotic-induced and polymorphonuclear leukocyte-mediated killing of Pseudomonas aeruginosa. Infect Immun. 1991 Jan; 59(1):302–8.

41. Krieg DP, Helmke RJ, German VF, Mangos JA. Resistance of mucoid Pseudomonas aeruginosa to nonopsonic phagocytosis by alveolar macrophages in vitro. Infect Immun. 1988 Dec; 56(12):3173–9.

42. Patel, J. D., Krupka, T. & Anderson, J. M. iNOS-mediated generation of reactive oxygen and nitrogen species by biomaterial-adherent neutrophils. J. Biomed. Mater. Res. A. 80, 381–390 (2007).

43. Zimmerli, W., Waldvogel, F. A., Vaudaux, P. & Nydeggerm, U. E. Pathogenesis of foreign body infection: description and characteristics of an animal model. J. Infect. Dis. 146, 487–497 (1982).

44. Franz, S., Rammelt, S., Scharnweber, D. & Simon, J. C. Immune responses to implants – a review of the implications for the design of immunomodulatory biomaterials. Biomaterials 32, 6692–6709 (2011).

45. Walker TS, Tomlin KL, Worthen GS, Poch KR, Lieber JG, Saavedra MT, Fessler MB, Malcolm KC, Vasil ML, Nick JA. Enhanced Pseudomonas aeruginosa biofilm development mediated by human neutrophils. Infect Immun. 2005 Jun; 73(6):3693–701.

46. K. Tam, V.J. Torres, Staphylococcus aureus secreted toxins and extracellular enzymes, Gram-Positive Pathogens, (2019) 640–668.

47. Kusch H, Engelmann S. Secrets of the secretome in Staphylococcus aureus. Int J Med Microbiol. 2014*; 304(2):*133–41.

48. A. Ross, H.W. Shoff, Staphylococcal scalded skin syndrome, StatPearls [Internet], (2020).

49. Nishifuji H, Sugai M, Amagai, M. Staphylococcal exfoliative toxins: molecular scissors of bacteria that attack the cutaneous defense barrier in mammals. J Dermatol. Sci. 49:21–31, 2008.

50. Bukowski M, Wladyka B, Dubin G. Exfoliative toxins of Staphylococcus aureus. Toxins (Basel). 2010 May*; 2(5):*1148–65.

51. Crosby HA, Kwiecinski J, Horswill AR. Staphylococcus aureus Aggregation and Coagulation Mechanisms, and Their Function in Host-Pathogen Interactions. Adv Appl Microbiol. 2016;96:1–41. https://doi.org/10.1016/bs.aambs.2016.07.018

52. K. Shettigar, T.S. Murali, Virulence factors and clonal diversity of Staphylococcus aureus in colonization and wound infection with emphasis on diabetic foot infection, European Journal of Clinical Microbiology & Infectious Diseases, (2020) 1–12.

53. Nygaard TK, Pallister KB, DuMont AL, DeWald M, Watkins RL, Pallister EQ, Malone C, Griffith S, Horswill AR, Torres VJ, Voyich JM. Alpha-toxin induces programmed cell death of human T cells, B cells, and monocytes during USA300 infection. PLoS One. 2012*; 7(5):e36532.*

54. Berube BJ, Bubeck Wardenburg J. Staphylococcus aureus α-toxin: nearly a century of intrigue. Toxins (Basel). 2013 Jun*; 5(6):*1140–66.

55. Wilke GA, Bubeck Wardenburg J. Role of a disintegrin and metalloprotease 10 in Staphylococcus aureus alpha-hemolysin-mediated cellular injury. Proc Natl Acad Sci U S A. 2010 Jul 27*; 107(30):*13473–8.

56. Inoshima I, Inoshima N, Wilke GA, Powers ME, Frank KM, Wang Y, Bubeck Wardenburg J. A Staphylococcus aureus pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. Nat Med. 2011 Sep 18*; 17(10):*1310–4.

57. Gill DM. Bacterial toxins: a table of lethal amounts. Microbiol Rev. 1982 Mar*; 46(1):*86–94.

58. Alonzo F 3rd, Torres VJ. The bicomponent pore-forming leucocidins of Staphylococcus aureus. Microbiol Mol Biol Rev. 2014 Jun*; 78(2):*199–230.

59. Koop G, Vrieling M, Storisteanu DM, Lok LS, Monie T, van Wigcheren G, Raisen C, Ba X, Gleadall N, Hadjirin N, Timmerman AJ, Wagenaar JA, Klunder HM, Fitzgerald JR, Zadoks R, Paterson GK, Torres C, Waller AS, Loeffler A, Loncaric I, Hoet AE, Bergström K, De Martino L, Pomba C, de Lencastre H, Ben Slama K, Gharsa H, Richardson EJ, Chilvers ER, de Haas C, van Kessel K, van Strijp JA, Harrison EM, Holmes MA. Identification of LukPQ, a novel, equid-adapted leukocidin of Staphylococcus aureus. *Sci Rep.* 2017 Jan 20*; 7():40660.*

60. Gravet A, Colin DA, Keller D, Girardot R, Monteil H, Prévost G. Characterization of a novel structural member, LukE-LukD, of the bi-component staphylococcal leucotoxins family. FEBS Lett. 1998 Oct 2*; 436(2):*202–8.

61. Reyes-Robles T, Lubkin A, Alonzo F 3rd, Lacy DB, Torres VJ. Exploiting dominant-negative toxins to combat Staphylococcus aureus pathogenesis. EMBO Rep. 2016 Mar*; 17(3):*428–40.

62. G.Y. Cheung, J.S. Bae, M. Otto, Pathogenicity and virulence of Staphylococcus aureus, Virulence, 12 (2021) 547–569.

63. A.T. Tromp, J.A. van Strijp, Studying staphylococcal leukocidins: a challenging endeavor, Frontiers in microbiology, 11 (2020) 611.

64. A.N. Spaan, J.A. van Strijp, V.J. Torres, Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors, Nature Reviews Microbiology, 15 (2017) 435.

65. Yanai M, Rocha MA, Matolek AZ, Chintalacharuvu A, Taira Y, Chintalacharuvu K, Beenhouwer DO. Separately or combined, LukG/LukH is functionally unique compared to other staphylococcal bicomponent leukotoxins. PLoS One. 2014*; 9(2):e89308.*

66. M. Baldry, M.S. Bojer, Z. Najarzadeh, M. Vestergaard, R.L. Meyer, D.E. Otzen, H. Ingmer, Phenol-Soluble Modulins Modulate Persister Cell Formation in Staphylococcus aureus, Frontiers in microbiology, 11 (2020).

67. Spaulding AR, Salgado-Pabón W, Kohler PL, Horswill AR, Leung DY, Schlievert PM. Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev. 2013 Jul; 26(3):422–47.

68. Falugi F, Kim HK, Missiakas DM, Schneewind O. Role of protein A in the evasion of host adaptive immune responses by Staphylococcus aureus. mBio. 2013 Aug 27; 4(5):e00575–13.

69. Kim JH, Lee J, Park J, Gho YS. Gram-negative and Gram-positive bacterial extracellular vesicles. Semin Cell Dev Biol. 2015 Apr; 40():97–104.

70. X. Wang, W.J. Eagen, J.C. Lee, Orchestration of human macrophage NLRP3 inflammasome activation by Staphylococcus aureus extracellular vesicles, Proceedings of the National Academy of Sciences, 117 (2020) 3174–3184.

71. Hong SW, Choi EB, Min TK, Kim JH, Kim MH, Jeon SG, Lee BJ, Gho YS, Jee YK, Pyun BY, Kim YK. An important role of α-hemolysin in extracellular vesicles on the development of atopic dermatitis induced by Staphylococcus aureus. PLoS One. 2014; 9(7):e100499.

72. Jun SH, Lee JH, Kim SI, Choi CW, Park TI, Jung HR, Cho JW, Kim SH, Lee JC. Staphylococcus aureus-derived membrane vesicles exacerbate skin inflammation in atopic dermatitis. Clin Exp Allergy. 2017 Jan; 47(1):85-96.

73. X. Wang, P.F. Koffi, O.F. English, J.C. Lee, Staphylococcus aureus Extracellular Vesicles: A Story of Toxicity and the Stress of 2020, Toxins, 13 (2021) 75.

74. M.F. Moradali, S. Ghods, B.H. Rehm, Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence, Frontiers in cellular and infection microbiology, 7 (2017) 39.

75. E.R. Green, J. Mecsas, Bacterial secretion systems: an overview, Virulence mechanisms of bacterial pathogens, (2016) 213-239.

76. S.P. Diggle, M. Whiteley, Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat, Microbiology, 166 (2020) 30.

77. A.O. Azghani, Pseudomonas aeruginosa and epithelial permeability: role of virulence factors elastase and exotoxin A, American journal of respiratory cell and molecular biology, 15 (1996) 132-140.

78. R.T. Pena, L. Blasco, A. Ambroa, B. González-Pedrajo, L. Fernández-García, M. López, I. Bleriot, G. Bou, R. García-Contreras, T.K. Wood, Relationship between quorum sensing and secretion systems, Frontiers in microbiology, 10 (2019) 1100.

79. C. Lombardi, J. Tolchard, S. Bouillot, L. Signor, C. Gebus, D. Liebl, D. Fenel, J.-M. Teulon, J. Brock, B. Habenstein, Structural and functional characterization of the type three secretion system (T3SS) needle of Pseudomonas aeruginosa, Frontiers in microbiology, 10 (2019) 573.

80. G. Horna, C. Amaro, A. Palacios, H. Guerra, J. Ruiz, High frequency of the exoU+/exoS+ genotype associated with multidrug-resistant "high-risk clones" of Pseudomonas aeruginosa clinical isolates from Peruvian hospitals, Scientific Reports, 9 (2019) 1-13.

81. W.P. Smith, M. Brodmann, D. Unterweger, Y. Davit, L.E. Comstock, M. Basler, K.R. Foster, The evolution of tit-for-tat in bacteria via the type VI secretion system, Nature communications, 11 (2020) 1-11.

82. M. Redero, C. López-Causapé, J. Aznar, A. Oliver, J. Blázquez, A.I. Prieto, Susceptibility to R-pyocins of Pseudomonas aeruginosa clinical isolates from cystic fibrosis patients, Journal of Antimicrobial Chemotherapy, 73 (2018) 2770-2776.

83. Y. Wang, D.T. Graves, Keratinocyte Function in Normal and Diabetic Wounds and Modulation by FOXO1, Journal of Diabetes Research, 2020 (2020).

84. R.M. Kishk, M.O. Abdalla, A.A. Hashish, N.A. Nemr, N. El Nahhas, S. Alkahtani, M.M. Abdel-Daim, S.M. Kishk, Efflux MexAB-Mediated Resistance in P. aeruginosa Isolated from Patients with Healthcare Associated Infections, Pathogens, 9 (2020) 471.

85. L. Zulianello, C. Canard, T. Köhler, D. Caille, J.-S. Lacroix, P. Meda, Rhamnolipids are virulence factors that promote early infiltration of primary human airway epithelia by Pseudomonas aeruginosa, Infection and immunity, 74 (2006) 3134-3147.

86. P.M. Alves, E. Al-Badi, C. Withycombe, P.M. Jones, K.J. Purdy, S.E. Maddocks, Interaction between Staphylococcus aureus and Pseudomonas aeruginosa is beneficial for colonisation and pathogenicity in a mixed biofilm, Pathogens and disease, 76 (2018) fty003.

87. R.A. Mendoza, J. Hsieh, R.D. Galiano, The impact of biofilm formation on wound healing, Wound healing-current perspectives, 10 (2019).

88. A.J. Rocha, M.R.d.O. Barsottini, R.R. Rocha, M.V. Laurindo, F.L.L.d. Moraes, S.L.d. Rocha, Pseudomonas aeruginosa: virulence factors and antibiotic resistance genes, Brazilian Archives of Biology and Technology, 62 (2019).

89. G. Golovkine, E. Reboud, P. Huber, Pseudomonas aeruginosa takes a multi-target approach to achieve junction breach, Frontiers in cellular and infection microbiology, 7 (2018) 532.

90. M. Garcia, E. Morello, J. Garnier, C. Barrault, M. Garnier, C. Burucoa, J.-C. Lecron, M. Si-Tahar, F.-X. Bernard, C. Bodet, Pseudomonas aeruginosa flagellum is critical for invasion, cutaneous persistence and induction of inflammatory response of skin epidermis, Virulence, 9 (2018) 1163-1175.

91. C.M. Suligoy, R.E. Díaz, A.-K. Gehrke, N. Ring, G. Yebra, J. Alves, M.I. Gómez, S. Wendler, J.R. Fitzgerald, L. Tuchscherr, Acapsular Staphylococcus aureus with a non-functional agr regains capsule expression after passage through the bloodstream in a bacteremia mouse model, Scientific Reports, 10 (2020) 1-12.

92. Broekhuizen CA, de Boer L, Schipper K, Jones CD, Quadir S, Vandenbroucke-Grauls CM, Zaat SA. Staphylococcus epidermidis is cleared from biomaterial implants but persists in peri-implant tissue in mice despite rifampicin/vancomycin treatment. J Biomed Mater Res A. 2008 May*;* 85*(2):*498–505.

93. Hamza T, Li B. Differential responses of osteoblasts and macrophages upon Staphylococcus aureus infection. *BMC Microbiol. 2014 Jul 25; 14():207.*

94. Campoccia, D. et al. Orthopedic implant infections: incompetence of *Staphylococcus epidermidis*. *Staphylococcus lugdunensis*, and *Enterococcus faecalis* to invade osteoblasts. *J. Biomed. Mater. Res.* A 104, 788–801 (2016).

95. Kubica M, Guzik K, Koziel J, Zarebski M, Richter W, Gajkowska B, Golda A, Maciag-Gudowska A, Brix K, Shaw L, Foster T, Potempa J. Potential new pathway for Staphylococcus aureus dissemination: the silent survival of S. aureus phagocytosed by human monocyte-derived macrophages. *PLoS One. 2008 Jan 9; 3(1):e1409.*

96. Wen Q, Gu F, Sui Z, Su Z, Yu T. The Process of Osteoblastic Infection by *Staphylococcus Aureus*. *Int J Med Sci*. 2020;17(10):1327–1332. Published 2020 May 29. https://doi.org/10.7150/ijms.45960

97. Foster TJ, Geoghegan JA, Ganesh VK, Höök M. Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol. 2014 Jan*;* 12*(1):*49–62.

98. Reilly, S. S., Hudson, M. C., Kellam, J. F. & Ramp, W. K. In vivo internalization of *Staphylococcus aureus* by embryonic chick osteoblasts. Bone 26, 63–70 (2000).

99. Hamza, T. et al. Intra-cellular *Staphylococcus aureus* alone causes infection in vivo. Eur. Cell. Mater. 25, 341–350 (2013).

100. Bui, L. M., Conlon, B. P. & Kidd, S. P. Antibiotic tolerance and the alternative lifestyles of *Staphylococcus aureus*. Essays Biochem. 61, 71–79 (2017).

101. Proctor, R. A. et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat. Rev. Microbiol. 4, 295–305 (2006).

102. Tuchscherr, L. et al. *Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence. J. Infect. Dis. 202, 1031–1040 (2010).

103. Tuchscherr L, Kreis CA, Hoerr V, Flint L, Hachmeister M, Geraci J, Bremer-Streck S, Kiehntopf M, Medina E, Kribus M, Raschke M, Pletz M, Peters G, Löffler B. Staphylococcus

aureus develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis. J Antimicrob Chemother. 2016 Feb*;* 71*(2):*438–48.

104. Zhou K, Li C, Chen D, Pan Y, Tao Y, Qu W, Liu Z, Wang X, Xie S. A review on nanosystems as an effective approach against infections of *Staphylococcus aureus*. *Int J Nanomedicine. 2018; 13():7333–7347.*

105. Marriott I, Gray DL, Rati DM, Fowler VG Jr, Stryjewski ME, Levin LS, Hudson MC, Bost KL. Osteoblasts produce monocyte chemoattractant protein-1 in a murine model of Staphylococcus aureus osteomyelitis and infected human bone tissue. Bone. 2005 Oct*;* 37*(4):*504–12.

106. Somayaji SN, Ritchie S, Sahraei M, Marriott I, Hudson MC. Staphylococcus aureus induces expression of receptor activator of NF-kappaB ligand and prostaglandin E2 in infected murine osteoblasts. Infect Immun. 2008 Nov*;* 76*(11):*5120–6.

107. Josse, J., Velard, F. & Gangloff, S. C. Staphylococcus aureus versus osteoblast: relationship and consequences in osteomyelitis. Front. Cell. Infect. Microbiol. https://doi.org/10.3389/fcimb.2015.00085 (2015).

108. de Mesy Bentley KL, Trombetta R, Nishitani K, Bello-Irizarry SN, Ninomiya M, Zhang L, Chung HL, McGrath JL, Daiss JL, Awad HA, Kates SL, Schwarz EM. Evidence of Staphylococcus Aureus Deformation, Proliferation, and Migration in Canaliculi of Live Cortical Bone in Murine Models of Osteomyelitis. J Bone Miner Res. 2017 May*;* 32*(5):*985–990.

109. Stones DH, Krachler AM. Against the tide: the role of bacterial adhesion in host colonization. Biochem Soc Trans. 2016 Dec 15;44(6):1571–1580. https://doi.org/10.1042/BST20160186. PMID: 27913666; PMCID: PMC5134996.

110. van Brakel R, Cune MS, van Winkelhoff AJ, de Putter C, Verhoeven JW, van der Reijden W. Early bacterial colonization and soft tissue health around zirconia and titanium abutments: an in vivo study in man. Clin Oral Implants Res. 2011 Jun*;* 22*(6):*571–7.

111. Bundy KJ, Butler MF, Hochman RF. An investigation of the bacteriostatic properties of pure metals. J Biomed Mater Res. 1980 Sep*;* 14*(5):*653–63.

112. Pollitt EJ, Crusz SA, Diggle SP. Staphylococcus aureus forms spreading dendrites that have characteristics of active motility. *Sci Rep*. 2015;5:17698. Published 2015 Dec 18. https://doi.org/10.1038/srep17698

113. Ubbink J, Schar-Zammaretti P. Colloidal properties and specific interactions of bacterial surfaces. Curr Opin Colloid Interface Sci. 12(4) (2007):263–270.

114. An YH, Friedman RJ. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res. 1998 Fall*;* 43*(3):*338–48.

115. Paharik, A. E. & Horswill, A. R. The Staphylococcal biofilm: adhesins, regulation, and host response. Microbiol. Spectr. 4, 2 (2016).

116. Kline, K. A., Fälker, S., Dahlberg, S., Normark, S. & Henriques-Normark, B. Bacterial adhesins in host-microbe interactions. Cell Host Microbe. 5, 580–592 (2009).

117. Boland, T., Latour, R. A. & Stutzenberger, F. J. in Handbook of bacterial adhesion: principles, methods, and applications (eds An, Y. H. & Friedman, R. J.) 1–27 (Humana Press, 2000).

118. Foster, T. J., Geoghegan, J. A., Ganesh, V. K. & Höök, M. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. Nat. Rev. Microbiol. 12, 49–62 (2014).

119. R. Wolden, M. Pain, R. Karlsson, A. Karlsson, E.G. Aarag Fredheim, J.P. Cavanagh, Identification of surface proteins in a clinical Staphylococcus haemolyticus isolate by bacterial surface shaving, BMC microbiology, 20 (2020) 1–18.

120. Arciola CR, Campoccia D, Montanaro L. Implant infections: adhesion, biofilm formation and immune evasion. Nature Review Microbiol. 16, 397–409, 2018.

121. Meenan NA, Visai L, Valtulina V, Schwarz-Linek U, Norris NC, Gurusiddappa S, Höök M, Speziale P, Potts JR. The tandem beta-zipper model defines high affinity fibronectin-binding repeats within Staphylococcus aureus FnBPA. J Biol Chem. 2007 Aug 31*;* 282*(35):*25893–902.

122. Eierhoff T, Bastian B, Thuenauer R, Madl J, Audfray A, Aigal S, Juillot S, Rydell GE, Müller S, de Bentzmann S, Imberty A, Fleck C, Römer W. A lipid zipper triggers bacterial invasion. Proc Natl Acad Sci U S A. 2014 Sep 2*; 111(35):*12895–900.

123. P. Speziale, G. Pietrocola, The multivalent role of fibronectin-binding proteins A and B (FnBPA and FnBPB) of Staphylococcus aureus in host infections, Frontiers in microbiology, 11 (2020) 2054.

124. Li X, Wang X, Thompson CD, Park S, Park WB, Lee JC. Preclinical Efficacy of Clumping Factor A in Prevention of Staphylococcus aureus Infection. *mBio*. 2016;7(1):e02232–15. Published 2016 Feb 2. https://doi.org/10.1128/mBio.02232-15

125. G. Loss, P.M. Simões, F. Valour, M.F. Cortês, L. Gonzaga, M. Bergot, S. Trouillet-Assant, J. Josse, A. Diot, E. Ricci, Staphylococcus aureus small colony variants (SCVs): news from a chronic prosthetic joint infection, Frontiers in cellular and infection microbiology, 9 (2019) 363.

126. Kim HK, Cheng AG, Kim HY, Missiakas DM, Schneewind O. Nontoxigenic protein A vaccine for methicillin-resistant Staphylococcus aureus infections in mice. J Exp Med. 2010 Aug 30*; 207(9):*1863–70.

127. Katsikogianni M, Spiliopoulou I, Dowling DP, Missirlis YF. Adhesion of slime producing Staphylococcus epidermidis strains to PVC and diamond-like carbon/silver/fluorinated coatings. J Mater Sci Mater Med. 2006 Aug*; 17(8):*679–89.

128. Liu Y, Tay JH. The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge. Water Res. 2002 Apr*; 36(7):*1653–65.

129. Mohamed N, Rainier TR Jr, Ross JM. Novel experimental study of receptor-mediated bacterial adhesion under the influence of fluid shear. Biotechnol Bioeng. 2000 Jun 20*; 68(6):*628–36.

130. Gallo J, Panacek A, Prucek R, Kriegova E, Hradilova S, Hobza M, et al. Silver nanocoating technology in the prevention of prosthetic joint infection. Materials. 9 (2016):337.

131. Martinez-Perez M, Perez-Jorge C, Lozano D, Portal-Nuñez S, Perez-Tanoira R, Conde A, Arenas M A, Hernandez-Lopez J M, de Damborenea J J, Gomez-Barrena E, Esbrit P, Esteban J. Evaluation of bacterial adherence of clinical isolates of *Staphylococcus sp.* using a competitive model: An in vitro approach to the "race for the surface" theory. Bone & Joint Research. 2017 May*; 6:5,* 315–322

132. Sanders D L, Kingsnorth A N, Lambie J., Bond P, Moate R, Steer J A. An experimental study exploring the relationship between the size of bacterial inoculum and bacterial adherence to prosthetic mesh. Surg Endosc. 2013 Mar*; 27,* 978–985.

133. Chu L, Yang Y, Yang S, Fan Q., Yu Z, Hu X L, James T D, He X P, Tang T. Preferential Colonization of Osteoblasts Over Co-cultured Bacteria on a Bifunctional Biomaterial Surface. Frontiers in microbiology. 2018 Oct*;* 9, 2219.

134. Busalmen JP, de Sánchez SR. Influence of pH and ionic strength on adhesion of a wild strain of Pseudomonas sp. to titanium. J Ind Microbiol Biotechnol. 2001 May*;*26(5):303–8.

135. Hamadi F, Latrache H, Mabrrouki M, Elghmari A, Outzourhit A, Ellouali M, Chtaini A. Effect of pH on distribution and adhesion of Staphylococcus aureus to glass, Journal of Adhesion Science and Technology. 2005 Apr*; 19:1,* 73–85.

136. Wilton M, Charron-Mazenod L, Moore R, Lewenza S. Extracellular DNA Acidifies Biofilms and Induces Aminoglycoside Resistance in Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy. 2015 Nov*; 60(1):* 544–553.

137. Kawarai T, Narisawa N, Suzuki Y, Nagasawa R, Senpuku H. Streptococcus mutans biofilm formation is dependent on extracellular DNA in primary low pH conditions. J Oral Biosci. 2016 Dec*;* 58:55–61.

138. Khelissa S O, Jama C, Abdallah M, Boukherroub R, Faille C, Chihib N E. Effect of incubation duration, growth temperature, and abiotic surface type on cell surface properties, adhesion and pathogenicity of biofilm-detached Staphylococcus aureus cells. AMB Express. 2017 Oct*; 7(1),* 191.

139. Abdallah M, Benoliel C, Jama C, Drider D, Dhulster P, Chihib NE. Thermodynamic prediction of growth temperature dependence in the adhesion of Pseudomonas aeruginosa and Staphylococcus aureus to stainless steel and polycarbonate. J Food Prot. 2014 Jul;77(7):1116–26.

140. Pavlovsky L, Sturtevant RA, Younger JG, Solomon MJ. Effects of temperature on the morphological, polymeric, and mechanical properties of Staphylococcus epidermidis bacterial biofilms. Langmuir. 2015 Feb; 17;31(6):2036–42.

141. Chen G, Das S. Electrostatics of soft charged interfaces with pH-dependent charge density: effect of consideration of appropriate hydrogen ion concentration distribution. RSC Adv. 5 (2015):4493–4501.

142. Gharechahi M, Moosavi H, Forghani M. Effect of surface roughness and materials composition on biofilm formation. J Biomater Nanobiotechnol. 3(4A) (2012):541–546.

143. Almousa R, Wen X, Na S, Anderson G, Xie D. A modified polyvinylchloride surface with antibacterial and antifouling functions. Poly Adv Tech. 30(5):1216–1225, 2016.

144. Ishihama, H., Ishii, K., Nagai, S. et al. An antibacterial coated polymer prevents biofilm formation and implant-associated infection. Sci Rep 11, 3602 (2021). https://doi.org/10.1038/s41598-021-82992-w

145. Oh JK, Yegin Y, Yang F, Xhang M, Li J, Huang S, et al. The influence of surface chemistry on the kinetics and thermodynamics of bacterial adhesion. Sci Rep. 8(1) (2018):17247.

146. Katsikogianni, M.G., Missirlis, Y.F. Bacterial adhesion onto materials with specific surface chemistries under flow conditions. J Mater Sci: Mater Med 21, 963–968 (2010). https://doi.org/10.1007/s10856-009-3975-y

147. MacKintosh EE, Patel JD, Marchant RE, Anderson JM. Effects of biomaterial surface chemistry on the adhesion and biofilm formation of Staphylococcus epidermidis in vitro. J Biomed Mater Res A. 2006 Sep 15;78(4):836–42. https://doi.org/10.1002/jbm.a.30905. PMID: 16817192.

148. Martinez, M.A.F., Balderrama, Í., Karam, P.S.B.H. et al. Surface roughness of titanium disks influences the adhesion, proliferation and differentiation of osteogenic properties derived from human. Int J Implant Dent 6, 46 (2020). https://doi.org/10.1186/s40729-020-00243-5

149. Dantas LC, da Silva-Neto JP, Dantas TS, Naves LZ, das Neves FD, da Mota AS. Bacterial Adhesion and Surface Roughness for Different Clinical Techniques for Acrylic Polymethyl Methacrylate. Int J Dent. 2016;2016:8685796. https://doi.org/10.1155/2016/8685796. Epub 2016 Jul 19. PMID: 27516775; PMCID: PMC4969518.

150. Li Mei, Henk J. Busscher, Henny C. van der Mei, Yijin Ren. Influence of surface roughness on streptococcal adhesion forces to composite resins. Dental Materials, Volume 27, Issue 8, 2011, Pages 770–778

151. Wu S, Zhang B, Liu Y, Suo X, Li H. Influence of surface topography on bacterial adhesion: A review (Review). Biointerphases. 2018 Nov 27;13(6):060801. https://doi.org/10.1116/1.5054057. PMID: 30482024.

152. Tripathy A, Sen P, Su B, Briscoe WH. Natural and bioinspired nanostructured bactericidal surfaces. Adv Colloid Interface Sci. 2017 Oct; 248:85–104. https://doi.org/10.1016/j.cis.2017.07.030. Epub 2017 Jul 27. PMID: 28780961; PMCID: PMC6643001.

153. Ivanova EP, Hasan J, Webb HK, Truong VK, Watson GS, Watson JA, Baulin VA, Pogodin S, Wang JY, Tobin MJ, Löbbe C, Crawford RJ. Natural bactericidal surfaces: mechanical rupture of Pseudomonas aeruginosa cells by cicada wings. Small. 2012 Aug 20;8(16):2489–94. https://doi.org/10.1002/smll.201200528. Epub 2012 Jun 4. PMID: 22674670.

154. Kelleher SM, Habimana O, Lawler J, O' Reilly B, Daniels S, Casey E, Cowley A. Cicada Wing Surface Topography: An Investigation into the Bactericidal Properties of Nanostructural Features. ACS Appl Mater Interfaces. 2016 Jun 22;8(24):14966–74. https://doi.org/10.1021/acsami.5b08309. Epub 2015 Nov 9. PMID: 26551558.

155. Jenkins J, Mantell J, Neal C, Gholinia A, Verkade P, Nobbs AH, Su B. Antibacterial effects of nanopillar surfaces are mediated by cell impedance, penetration and induction of oxidative

stress. Nat Commun. 2020 Apr 2;11(1):1626. https://doi.org/10.1038/s41467-020-15471-x. PMID: 32242015; PMCID: PMC7118135.

156. Dundar Arisoy F, Kolewe KW, Homyak B, Kurtz IS, Schiffman JD, Watkins JJ. Bioinspired Photocatalytic Shark-Skin Surfaces with Antibacterial and Antifouling Activity via Nanoimprint Lithography. ACS Appl Mater Interfaces. 2018 Jun 13;10(23):20055–20063. https://doi.org/10.1021/acsami.8b05066. Epub 2018 Jun 1. PMID: 29790348; PMCID: PMC6013830.

157. Stewart, P. S. (2003). Diffusion in biofilms. Journal of bacteriology, 185(5), 1485–1491.

158. Jasper Wille, Tom Coenye. Biofilm dispersion: The key to biofilm eradication or opening Pandora's box? Biofilm, Volume 2, 2020, 100027, ISSN 2590-2075, https://doi.org/10.1016/j.bioflm.2020.100027.

159. Kolpen, M., Hansen, C. R., Bjarnsholt, T., Moser, C., Christensen, L. D., van Gennip, M., & Jensen, P. Ø. (2010). Polymorphonuclear leucocytes consume oxygen in sputum from chronic Pseudomonas aeruginosa pneumonia in cystic fibrosis. Thorax, 65(1), 57–62.

160. Ryan C. Hunter, Terry J. Beveridge. Application of a pH-Sensitive Fluoroprobe (C-SNARF-4) for pH Microenvironment Analysis in *Pseudomonas aeruginosa* Biofilms. Applied and Environmental Microbiology May 2005, 71 (5) 2501 2510; https://doi.org/10.1128/AEM.71.5.2501-2510.2005

161. Dominika T. Gruszka, Justyna A. Wojdyla, Richard J. Bingham, Johan P. Turkenburg, Iain W. Manfield, Annette Steward, Andrew P. Leech, Joan A. Geoghegan, Timothy J. Foster, Jane Clarke, Jennifer R. Potts. Structure of a biofilm-forming protein. Proceedings of the National Academy of Sciences Apr 2012, 109 (17) E1011–E1018; https://doi.org/10.1073/pnas.1119456109

162. C. Dotto, A.L. Serrat, M. Ledesma, C. Vay, M. Ehling-Schulz, D.O. Sordelli, T. Grunert, F. Buzzola, Salicylic acid stabilizes Staphylococcus aureus biofilm by impairing the agr quorum-sensing system, Scientific Reports, 11 (2021) 1–14.

163. P.V. Bramhachari, Implication of quorum sensing system in biofilm formation and virulence, Springer, 2019.

164. Y. Jiang, M. Geng, L. Bai, Targeting biofilms therapy: current research strategies and development hurdles, Microorganisms, 8 (2020) 1222.

165. M. Otto, Staphylococcal biofilms, Bacterial biofilms, (2008) 207–228.

166. M.R. Bennett, I.P. Thomsen, Epidemiological and Clinical Evidence for the Role of Toxins in S. aureus Human Disease, Toxins, 12 (2020) 408.

167. Roberts I S. The biochemistry and genetics of capsular polysaccharide production in bacteria. Ann Rev Microbiol. 50285315, 1996

168. D. Bittersuermann, Influence of bacterial polysialic capsules on host defense-masquerade and mimicry. Polysialic Acid, Birkhauser, Basel, 1993.

169. N. Bhasin, A. Albus, F. Michon, P.J. Livolsi, J.S. Park, J.C. Lee, Identification of a gene essential for O-acetylation of the Staphylococcus aureus type 5 capsular polysaccharide, Molecular microbiology, 27 (1998) 9–21.

170. A.O. Tzianabos, J.Y. Wang, J.C. Lee, Structural rationale for the modulation of abscess formation by Staphylococcus aureus capsular polysaccharides, Proceedings of the National Academy of Sciences, 98 (2001) 9365–9370.

171. D. Hilmi, M. Parcina, D. Stollewerk, J. Ostrop, M. Josten, A. Meilaender, U. Zaehringer, T.A. Wichelhaus, G. Bierbaum, K. Heeg, Heterogeneity of host TLR2 stimulation by Staphylocoocus aureus isolates, PloS one, 9 (2014) e96416.

172. C.R. Schaeffer, T.-M.N. Hoang, C.M. Sudbeck, M. Alawi, I.E. Tolo, D.A. Robinson, A.R. Horswill, H. Rohde, P.D. Fey, Versatility of biofilm matrix molecules in Staphylococcus epidermidis clinical isolates and importance of polysaccharide intercellular adhesin expression during high shear stress, Msphere, 1 (2016).

173. S.E. Cramton, C. Gerke, N.F. Schnell, W.W. Nichols, F. Götz, The intercellular adhesion (ica) locus is present in Staphylococcus aureus and is required for biofilm formation, Infection and immunity, 67 (1999) 5427–5433.

174. C.C. Formosa-Dague, C. Feuillie, A. Beaussart, S. Derclaye, S. Kucharikova, I.I. Lasa, P. Van Dijck, Y.F. Dufrene, Sticky matrix: adhesion mechanism of the staphylococcal polysaccharide intercellular adhesin, ACS nano, 10 (2016) 3443–3452.

175. H.T. Nguyen, T.H. Nguyen, M. Otto, The staphylococcal exopolysaccharide PIA–Biosynthesis and role in biofilm formation, colonization, and infection, Computational and Structural Biotechnology Journal, (2020).

176. T. Saba, M. Sajid, A.A. Khan, R. Zahra, Role of intracellular adhesion icaAD and agr genes in biofilm formation in clinical S. aureus isolates and assessment of two phenotypic methods, Pakistan journal of medical sciences, 34 (2018) 633.

177. D. Skurnik, C. Cywes-Bentley, G.B. Pier, The exceptionally broad-based potential of active and passive vaccination targeting the conserved microbial surface polysaccharide PNAG, Expert review of vaccines, 15 (2016) 1041–1053.

178. T.J. Foster, Surface Proteins of Staphylococcus epidermidis, Frontiers in microbiology, 11 (2020) 1829.

179. A.E. Yarawsky, S.L. Johns, P. Schuck, A.B. Herr, The biofilm adhesion protein Aap from Staphylococcus epidermidis forms zinc-dependent amyloid fibers, Journal of Biological Chemistry, 295 (2020) 4411–4427.

180. Brown S, Santa Maria JP Jr, Walker S. Wall teichoic acids of gram-positive bacteria. Annu Rev Microbiol. 2013;67:313–336. https://doi.org/10.1146/annurev-micro-092412-155620

181. Gautam S, Kim T, Lester E, Deep D, Spiegel DA. Wall teichoic acids prevent antibody binding to epitopes within the cell wall of Staphylococcus aureus. ACS Chem Biol. 2016;11(1):25–30. https://doi.org/10.1021/acschembio.5b00439

182. Kohler T, Weidenmaier C, Peschel A. Wall teichoic acid protects Staphylococcus aureus against antimicrobial fatty acids from human skin. J Bacteriol. 2009 Jul; 191(13):4482–4.

183. Holland LM, Conlon B, O'Gara JP. Mutation of tagO reveals an essential role for wall teichoic acids in Staphylococcus epidermidis biofilm development. Microbiology (Reading). 2011 Feb; 157(Pt 2):408–418.

184. D.M. Mrochen, L.M. Fernandes de Oliveira, D. Raafat, S. Holtfreter, Staphylococcus aureus Host Tropism and Its Implications for Murine Infection Models, International Journal of Molecular Sciences, 21 (2020) 7061.

185. R. van Dalen, A. Peschel, N.M. van Sorge, Wall teichoic acid in Staphylococcus aureus host interaction, Trends in microbiology, (2020).

186. Mukherjee, S., Bassler, B.L. Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol 17, 371–382 (2019). https://doi.org/10.1038/s41579-019-0186-5

187. J.K. Vasquez, H.E. Blackwell, Simplified autoinducing peptide mimetics with single-nanomolar activity against the Staphylococcus aureus AgrC quorum sensing receptor, ACS infectious diseases, 5 (2019) 484–492.

188. Le KY, Otto M. Quorum-sensing regulation in staphylococci-an overview. Front Microbiol. 2015;6:1174. Published 2015 Oct 27. https://doi.org/10.3389/fmicb.2015.01174

189. Traber KE, Lee E, Benson S, Corrigan R, Cantera M, Shopsin B, Novick RP. agr function in clinical Staphylococcus aureus isolates. Microbiology (Reading). 2008 Aug; 154(Pt 8):2265–2274.

190. Zhao L, Xue T, Shang F, Sun H, Sun B. Staphylococcus aureus AI-2 quorum sensing associates with the KdpDE two-component system to regulate capsular polysaccharide synthesis and virulence. Infect Immun. 2010 Aug; 78(8):3506–15.

191. Xue T, Zhao L, Sun B. LuxS/AI-2 system is involved in antibiotic susceptibility and autolysis in Staphylococcus aureus NCTC 8325. Int J Antimicrob Agents. 2013 Jan; 41(1):85–9.

192. M. Cerioli, C. Batailler, A. Conrad, S. Roux, T. Perpoint, A. Becker, C. Triffault-Fillit, S. Lustig, M.-H. Fessy, F. Laurent, Pseudomonas aeruginosa implant-associated Bone and Joint Infections: experience in a regional reference center in France, Frontiers in Medicine, 7 (2020) 701.

193. A. Soares, K. Alexandre, M. Etienne, Tolerance and Persistence of Pseudomonas aeruginosa in Biofilms Exposed to Antibiotics: Molecular Mechanisms, Antibiotic Strategies and Therapeutic Perspectives, Frontiers in microbiology, 11 (2020) 2057.

194. O'Toole GA. Flagellar and twitching motility are necessary for Pseudomonas aeruginosa biofilm development. Mol microbiol. 30, 1998295304

195. Semmler AB, Whitchurch CB, Mattick JS. A re-examination of twitching motility in Pseudomonas aeruginosa. Microbiology (Reading). 1999 Oct;145 (Pt 10):2863–73. https://doi.org/10.1099/00221287-145-10-2863. PMID: 10537208.

196. A. Devaraj, J.R. Buzzo, L. Mashburn-Warren, E.S. Gloag, L.A. Novotny, P. Stoodley, L.O. Bakaletz, S.D. Goodman, The extracellular DNA lattice of bacterial biofilms is structurally related to Holliday junction recombination intermediates, Proceedings of the National Academy of Sciences, 116 (2019) 25068–25077.

197. M.T.T. Thi, D. Wibowo, B.H. Rehm, Pseudomonas aeruginosa Biofilms, International Journal of Molecular Sciences, 21 (2020) 8671.

198. Jennings LK, Storek KM, Ledvina HE, Coulon C, Marmont LS, Sadovskaya I, Secor PR, Tseng BS, Scian M, Filloux A, Wozniak DJ, Howell L, Parsek MR. Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. PNAS. 112(36):11353–11358, 2015.

199. Jennings, L. K. et al. Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the *Pseudomonas aeruginosa* biofilm matrix. Proc. Natl Acad. Sci. USA 112, 11353–11358 (2015).

200. V.A. Ray, P.J. Hill, C.K. Stover, S. Roy, C.K. Sen, L. Yu, D.J. Wozniak, A. DiGiandomenico, Anti-Psl targeting of Pseudomonas aeruginosa biofilms for neutrophil-mediated disruption, Scientific Reports, 7 (2017) 1–12.

201. L.K. Jennings, J.E. Dreifus, C. Reichhardt, K.M. Storek, P.R. Secor, D.J. Wozniak, K.B. Hisert, M.R. Parsek, Pseudomonas aeruginosa aggregates in cystic fibrosis sputum produce exopolysaccharides that likely impede current therapies, Cell reports, 34 (2021) 108782.

202. N. Blanco-Cabra, B. Paetzold, T. Ferrar, R. Mazzolini, E. Torrents, L. Serrano, M. L. Luch-Senar, Characterization of different alginate lyases for dissolving Pseudomonas aeruginosa biofilms, Scientific Reports, 10 (2020) 1–10.

203. G.M. Matar, Pseudomonas and Acinetobacter: From drug resistance to pathogenesis, Frontiers in cellular and infection microbiology, 8 (2018) 68.

204. Borlee, B. R. et al. *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. Mol. Microbiol. 75, 827–842 (2010).

205. Reichhardt, C., Wong, C., Passos da Silva, D., Wozniak, D. J. & Parsek, M. R. CdrA interactions within the *Pseudomonas aeruginosa* biofilm matrix safeguard it from proteolysis and promote cellular packing. *MBio* 9, https://doi.org/10.1128/mBio.01376-18 (2018).

206. Tseng BS, Reichhardt C, Merrihew GE, Araujo-Hernandez SA, Harrison JJ, MacCoss MJ, Parsek MR. A Biofilm Matrix-Associated Protease Inhibitor Protects Pseudomonas aeruginosa from Proteolytic Attack. Bio. 2018 Apr 10*; 9(2):*

207. Zeng G, Vad BS, Dueholm MS, Christiansen G, Nilsson M, Tolker-Nielsen T, Nielsen PH, Meyer RL, Otzen DE. Functional bacterial amyloid increases Pseudomonas biofilm hydrophobicity and stiffness. *Front Microbiol. 2015; 6():1099.*

208. Adam, E. C., Mitchell, B. S., Schumacher, D. U., Grant, G. & Schumacher, U. *Pseudomonas aeruginosa* II lectin stops human ciliary beating: therapeutic implications of fucose. Am. J. Respir. Crit. care Med. 155, 2102–2104 (1997).

209. S.K. Saggu, G. Jha, P.C. Mishra, Enzymatic degradation of biofilm by metalloprotease from Microbacterium sp. SKS10, Frontiers in bioengineering and biotechnology, 7 (2019) 192.

210. S. Zheng, T. Eierhoff, S. Aigal, A. Brandel, R. Thuenauer, S. De Bentzmann, A. Imberty, W. Römer, The Pseudomonas aeruginosa lectin LecA triggers host cell signalling by glycosphingolipid-dependent phosphorylation of the adaptor protein CrkII, Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1864 (2017) 1236–1245.

211. Cassin EK, Tseng BS. Pushing beyond the Envelope: the Potential Roles of OprF in *Pseudomonas aeruginosa* Biofilm Formation and Pathogenicity. J Bacteriol. 2019 Aug 22;201(18):e00050–19. https://doi.org/10.1128/JB.00050-19. PMID: 31010902; PMCID: PMC6707909.

212. Song F, Wang H, Sauer K, Ren D. Cyclic-di-GMP and *oprF* Are Involved in the Response of *Pseudomonas aeruginosa* to Substrate Material Stiffness during Attachment on Polydimethylsiloxane (PDMS). *Front Microbiol. 2018; 9():110.*

213. E.K. Cassin, B.S. Tseng, Pushing beyond the envelope: the potential roles of OprF in Pseudomonas aeruginosa biofilm formation and pathogenicity, Journal of bacteriology, 201 (2019).

214. A. Tahrioui, R. Duchesne, E. Bouffartigues, S. Rodrigues, O. Maillot, D. Tortuel, J. Hardouin, L. Taupin, M.-C. Groleau, A. Dufour, Extracellular DNA release, quorum sensing, and PrrF1/ F2 small RNAs are key players in Pseudomonas aeruginosa tobramycin-enhanced biofilm formation, NPJ biofilms and microbiomes, 5 (2019) 1–11.

215. M.H. Muhammad, A.L. Idris, X. Fan, Y. Guo, Y. Yu, X. Jin, J. Qiu, X. Guan, T. Huang, Beyond risk: Bacterial biofilms and their regulating approaches, Frontiers in microbiology, 11 (2020) 928.

216. K.P. Rumbaugh, K. Sauer, Biofilm dispersion, Nature Reviews Microbiology, 18 (2020) 571–586.

217. Ute Römling, Michael Y. Galperin, Mark Gomelsky. Cyclic di-GMP: the First 25 Years of a Universal Bacterial Second Messenger. Microbiology and Molecular Biology Reviews Mar 2013, 77 (1) 1–52; https://doi.org/10.1128/MMBR.00043-12

218. N. Ramasubbu, L. Thomas, C. Ragunath, J. Kaplan, Structural analysis of dispersin B, a biofilm-releasing glycoside hydrolase from the periodontopathogen Actinobacillus actinomycetemcomitans, Journal of molecular biology, 349 (2005) 475–486.

219. Breyers JD. Modeling biofilm accumulation. Physiol Model Microbiol. Vol II, CRC Press. 109–144, 1988.

220. Kaplan JB. Biofilm dispersal: mechanisms, clinical implications, and potential therapeutic uses. J Dent Res. 2010;89(3):205–218. https://doi.org/10.1177/0022034509359403

221. Kaplan, J. B., Ragunath, C., Ramasubbu, N., & Fine, D. H. (2003). Detachment of Actinobacillus actinomycetemcomitans biofilm cells by an endogenous β-hexosaminidase activity. Journal of bacteriology, 185(16), 4693–4698.

222. Cherny, K. E., & Sauer, K. (2020). Untethering and degradation of the polysaccharide matrix are essential steps in the dispersion response of Pseudomonas aeruginosa biofilms. Journal of bacteriology, 202(3).

223. J.W. Lamppa, K.E. Griswold, Alginate lyase exhibits catalysis-independent biofilm dispersion and antibiotic synergy, Antimicrobial Agents and Chemotherapy, 57 (2013) 137–145.

224. Chambers, J. R., Cherny, K. E., & Sauer, K. (2017). Susceptibility of Pseudomonas aeruginosa dispersed cells to antimicrobial agents is dependent on the dispersion cue and class of the antimicrobial agent used. Antimicrobial agents and chemotherapy, 61(12).

225. Barraud, N., Hassett, D. J., Hwang, S. H., Rice, S. A., Kjelleberg, S., & Webb, J. S. (2006). Involvement of nitric oxide in biofilm dispersal of Pseudomonas aeruginosa. Journal of bacteriology, 188(21), 7344–7353.

226. Huynh, T. T., McDougald, D., Klebensberger, J., Al Qarni, B., Barraud, N., Rice, S. A., … & Schleheck, D. (2012). Glucose starvation-induced dispersal of Pseudomonas aeruginosa biofilms is cAMP and energy dependent. PLoS One, 7(8), e42874.

227. Donelli, G., Francolini, I., Romoli, D., Guaglianone, E., Piozzi, A., Ragunath, C., & Kaplan, J. B. (2007). Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. Antimicrobial agents and chemotherapy, 51(8), 2733–2740.

228. Darouiche, R. O., Mansouri, M. D., Gawande, P. V., & Madhyastha, S. (2009). Antimicrobial and antibiofilm efficacy of triclosan and DispersinB® combination. Journal of antimicrobial chemotherapy, 64(1), 88–93.

229. Waryah, C. B., Wells, K., Ulluwishewa, D., Chen-Tan, N., Gogoi-Tiwari, J., Ravensdale, J., …
    & Mukkur, T. (2017). In vitro antimicrobial efficacy of tobramycin against Staphylococcus
    aureus biofilms in combination with or without DNase I and/or dispersin B: a preliminary
    investigation. Microbial Drug Resistance, 23(3), 384–390.
230. Gawande, P. V., Clinton, A. P., LoVetri, K., Yakandawala, N., Rumbaugh, K. P., & Madhyastha,
    S. (2014). Antibiofilm efficacy of DispersinB® wound spray used in combination with a sil-
    ver wound dressing. *Microbiology insights*, *7*, MBI-S13914.
231. Moradali, M.F.; Ghods, S.; Rehm, B.H. Pseudomonas aeruginosa Lifestyle: A Paradigm for
    Adaptation, Survival, and Persistence. Front. Cell Infect. Microbiol. 2017, 7, 39.
232. R. García-Contreras, D. Loarca, C. Pérez-González, J.G. Jiménez-Cortés, A. Gonzalez-
    Valdez, G. Soberón-Chávez, Rhamnolipids stabilize quorum sensing mediated cooperation
    in Pseudomonas aeruginosa, FEMS microbiology letters, 367 (2020) fnaa080.
233. X. Qin, G.K. Thota, R. Singh, R. Balamurugan, F.M. Goycoolea, Synthetic homoserine
    lactone analogues as antagonists of bacterial quorum sensing, Bioorganic chemistry, 98
    (2020) 103698.
234. M. Kostylev, D.Y. Kim, N.E. Smalley, I. Salukhe, E.P. Greenberg, A.A. Dandekar, Evolution
    of the Pseudomonas aeruginosa quorum-sensing hierarchy, Proceedings of the National
    Academy of Sciences, 116 (2019) 7027–7032.
235. P. Thakur, N.K. Saini, V.K. Thakur, V.K. Gupta, R.V. Saini, A.K. Saini, Rhamnolipid the
    Glycolipid Biosurfactant: Emerging trends and promising strategies in the field of biotech-
    nology and biomedicine, Microbial Cell Factories, 20 (2021) 1–15.
236. Cornelis P. Putting an end to the Pseudomonas aeruginosa IQS controversy. Microbiologyopen.
    2020 Feb;9(2):e962. https://doi.org/10.1002/mbo3.962. Epub 2019 Oct 30. PMID: 31667921;
    PMCID: PMC7002111.

Chapter 3
Prevention of Infection: Best Practice and Novel Strategies

Aaron Jackson, Steven Yacovelli, and Javad Parvizi

Abstract Musculoskeletal infections are a devastating complication that can occur after total joint arthroplasty (TJA) procedures and are associated with increased patient mortality, increased length of stay, and healthcare costs (Whitehouse et al., Infect Control Hosp Epidemiol 23(04):183–189, 2002). With increased utilization of TJA as the treatment of choice for degenerative joint pathologies, an increase in periprosthetic joint infection (PJI) is expected in the future (Whitehouse et al., Infect Control Hosp Epidemiol 23(04):183–189, 2002). Annual costs associated with infections related to TJA are expected to be approximately \$1.62 billion by 2020 (Kurtz et al., J Arthroplasty 27(8):61.e1–65.e1, 2012). In addition to infection prevention, an abundance of research is underway with hopes of improving the ability to accurately diagnose and manage periprosthetic joint infections. Treating periprosthetic joint infections is a challenging process due to the complex nature of biofilms and microbial resistance. This chapter delves into various novel technologies that are currently in development, as well as developments in operating room etiquette, enhanced surgical techniques, implant surface modifications, and proper antibiotic use. Due to the inherent complexity of infectious disease, further partnership between clinicians and scientists is necessary to continue toward improved prevention and management of musculoskeletal infections.

Keywords Surgery · Risk · Infection · Prevention · Cleansing · Antimicrobials · Prophylaxis · Dressings · Biomaterials · Laminar flow

3.1 Introduction

Musculoskeletal infections are a devastating and potentially life-threateningcomplication associated with total joint arthroplasties (TJAs). Approximately 1–2% of TJAs will ultimately progress to peripheral joint infections (PJIs), with the most common cause of hospital readmissions being PJI [3]. PJIs

A. Jackson · S. Yacovelli · J. Parvizi (✉)
Rothman Orthopaedic Institute at Thomas Jefferson University, Philadelphia, PA, USA

lead to increased length of stay, risk of increased mortality, and increased healthcare costs. One study reported an average cost of $116,382 for total joint revisions due to infection, compared to $28,249 for primary TJAs [4]. To improve patient outcomes and prevent infections, it is imperative to take all measures necessary to identify any modifiable patient risk factors and utilize current evidence in order to make the best decisions for the patients. In the event of inevitable PJI, proper management begins with an accurate diagnosis, followed by successful treatment. Unfortunately, a gold standard diagnostic tool has yet to be identified.

There has been an abundance of research developed in an attempt to identify preventative measures for reducing the prevalence of musculoskeletal infections, such as using novel technologies to inhibit biofilm formation, proper operating room etiquette, enhanced surgical techniques, and proper antibiotic use. Despite the advancements in research and clinical practice, musculoskeletal infections remain a significant burden on the healthcare system.

3.2   Host Factors/Risk Mitigation

It is imperative to identify and manage any host risk factors, both modifiable and non-modifiable, as this may help in the prevention of future perioperative infections. Some modifiable risk factors that have been shown to increase risk of PJI and surgical site infections (SSIs) include smoking and alcohol use, elevated BMI (>40), and a history of diabetes mellitus. Non-modifiable risk factors, including male gender, increased age, and black ethnicity, have also been shown to increase risk of PJI/SSI [5].

Smoking has been known to be associated with a significantly increased risk of PJI and SSI [6]. Sørensen et al. found that smoking reduced the oxidative burst from neutrophils and monocytes by half when comparing smokers to nonsmokers and never smokers [7]. Oxidative burst is an effective mechanism used by these innate immune cells to eliminate phagocytized surgical bacteria, such as *Staphylococcus aureus* [8, 9]. They also noted a significant increase in neutrophilic and monocytic oxidative burst after 20 days of abstinence from smoking. Additionally, one systematic review showed smoking cessation 4–8 weeks prior to surgery significantly reduced SSIs by 50% [10].

When performing elective orthopedic procedures, pertinent host risk factors should be addressed to ensure the risk to the patient does not outweigh the benefits of the procedure. The following are absolute contraindications to surgery and must be managed prior to elective surgery [11]:

1. Serum glucose ≥200 mg/dl, active sepsis
2. Intra-articular injections within 3 months
3. Active intravenous drug use
4. Super obesity (BMI ≥50 kg/m^2)
5. Active joint infection

6. Untreated HIV

When assessing preoperative risk, it may be beneficial to utilize risk stratification tools, such as the Readmission Risk Assessment Tool (RRAT). The RRAT is used to predict the probability of readmission by calculating a value based on patient risk factors, for example, MRSA colonization, 3 points; BMI ≥40–3 points; and smoking, 1 point. A study by Boraiah et al. analyzed the relationship between RRAT scores and readmission after primary total hip and knee arthroplasty [12]. 45% of readmissions were found to be associated with surgical site infections, and the study found a significant association between RRAT score and readmission [11].

3.3 Surgical Technique and Surgical Site Preparation

3.3.1 Skin Cleansing

The CDC currently recommends preoperative skin cleansing at least one night prior to surgery to decrease skin cultures [13]. There are multiple skin cleansing agents that can be utilized including 2% or 4% chlorhexidine-gluconate (CHG)-coated products, isopropyl alcohol, bar soap, and povidone-iodine (PI). An additional study has demonstrated the benefit of using CHG and its ability to decrease skin flora preoperatively [14].

Although there is evidence to show a decrease in skin cultures after using chlorhexidine, there does not appear to be a significant reduction in SSI [15, 16]. Colling et al. found a decrease in SSI from *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) after preoperative shower and bath, but found there was no significant decrease in the total incidence of SSI [17]. Despite the inconsistent evidence on the effectiveness of preoperative skin cleansing, there are no studies to date that indicate a negative outcome of using an antiseptic protocol prior to surgery. The lack of consistent outcomes between studies may be a result of varying skin cleansing protocols between institutions and a patient non-compliance rate of approximately 78%, noted in a study by Kapadia et al. [18]. To improve patient compliance, it may be important to consider implementing a comprehensible skin cleansing protocol with thorough, clear instructions.

3.3.2 Hair Removal

In addition to preoperative skin cleansing, hair removal at the incision site is an additional procedure that is widely practiced in an attempt to further decrease risk of SSI. The intended reason for this procedure is to prevent hair from entering the incision site and possibly causing SSI. Despite this rationale, there is inconsistent evidence to show a reduction in SSI after preoperative hair removal when compared

to no hair removal [19]. There are three routine methods of hair removal which include electric clippers, shaving, and depilatory creams. Of these three methods, electric clippers and depilatory creams were found to have lower rates of SSI, when compared to shaving [20]. Shaving with a razorblade was found to be less efficacious secondary to the microabrasions on the skin caused by the razorblade, which can harbor infectious bacteria [21], and was shown to increase rates of SSI in some studies [18]. Although depilatory creams have been shown to have lower risk of SSI than clipping and shaving, depilatory creams may not be the most pragmatic approach for hair removal due to the increased wait times for depilation, cost, and the potential for a skin hypersensitivity reaction [20]. Timing of removal may be important as some evidence suggests removing hair as close to the operation as practically possible, ideally outside of the operating room, could limit unnecessary risk of wound contamination [22, 23]. Due to the lack of consistent evidence, it is recommended that preoperative hair removal be performed only when the presence of the hair will interfere with operation.

3.4 Operating Room Environment (Laminar Flow)/ Personnel

3.4.1 Laminar Flow

The operating room environment is an important factor to consider when attempting to minimize SSIs, particularly airborne bacteria, which can potentially land on the surgical site or sterile instruments and gowns, ultimately causing infection of the wound. Laminar air flow (LAF) systems have been utilized for decades to prevent the threat of airborne bacteria. LAFs provide highly filtered, continuous air flow moving at the same velocity in a uniform direction over the surface of surgical sites and sterile instruments in an attempt to prevent airborne bacterial contamination. Despite the promising mechanism behind LAF systems, there is continued debate on the efficacy of LAF systems in the operating room, which brings into question their cost-effectiveness. Evidence shows a significant increase in the building and operating costs of LAF systems, at 24% and 34%, respectively, compared to conventional operating rooms [24].

The lack of consistent conclusions in the current literature may be secondary to the variability of use between LAF systems. There are many different LAF systems available for use in the operating room (OR), all of which have varying configurations, such as air velocity, horizontal or vertical flow, etc. It is also known that the LAF systems only affect the area directly in the path of the laminar air flow and do not protect areas outside of this zone within the operating room [25]. In addition to variability in system configurations, some evidence suggests that poorly positioned operating room staff can create turbulence within the laminar air flow, potentially disrupting the removal of airborne bacteria over the sterile field [26]. To date, there

lack any large, well-controlled clinical trials to determine the efficacy of LAF systems. Current data suggests that patients may undergo surgical procedures in either conventional ORs or ORs containing state-of-the-art LAF systems without any increase risk of SSI [27].

3.4.2 Operating Room Traffic

Minimizing unnecessary personnel in the OR should be a priority, as increased foot traffic has been shown to increase the rate of SSI [28]. Bacterial shedding is another concern in the operating room environment. Evidence has demonstrated a rate of greater than 400 bacteria colony-forming units per square foot per hour in an operating room during surgery compared to 13 colony-forming units per square foot per hour in an empty operating room [29]. Door opening, associated with personnel entering and exiting the OR, has been linked to disturbing the laminar air flow, which may also increase risk of contamination of the wound from airborne pathogens [25]. Bedard et al. found, on average, 0.64 door openings per minute, during 100 observed total joint arthroplasty surgeries [30]. Overall, it is recommended that the leadership in the OR make it a priority to educate their staff on this modifiable risk and to only allow for critical staff to be present during operations.

3.4.3 Gowns

Wearing surgical gowns is known to reduce the amount of bacterial shedding from OR personnel during procedures. Throughout decades of research on the topic, there has yet to be a consensus on the type of gown that is the most efficacious at preventing bacterial contamination [10]. Ward et al. found disposable paper gowns to have less bacterial contamination on the surgeon's sleeves compared to using reusable cloth gowns [31]. It is believed that bacterial strike-through can occur at a much greater rate in the more porous reusable cloth gowns, especially when the gown becomes wet [30, 32]. On the contrary, Garibaldi et al. performed a prospective randomized controlled trial on nearly 500 patients and found that, regardless of wearing cotton poplin gowns or disposable gowns, there was no difference in intraoperative infection rates [33]. To date, there is no evidence to suggest that changing surgical gowns during prolonged surgical procedures will reduce SSIs.

Skin-borne pathogens on the hands of surgical staff are of great concern for potentially contaminating surgical sites. Wearing sterile gloves is common practice during surgical procedures and has been known to reduce intraoperative infections, as well as protect the surgical staff from potential disease exposure from the patient. However, the use of surgical gloves does not equate to impunity; glove perforation is a concern for surgeons in all subspecialties, and preventative measures should be taken to reduce such occurrences in the operating room. One study noted a glove

perforation rate as high as 26% in elective orthopedic surgery procedures [34]. Additionally, Mistelli et al. noted a significant increase in the occurrence of SSI after glove perforation when in the absence of surgical prophylaxis [35]. In a randomized controlled trial, assessing whether changing gloves at regular 20-minute intervals would decrease the rate of glove perforations and contamination during total hip arthroplasty procedures, this study found a significant decrease ($p < 0.05$) in both glove perforations and glove contamination when compared to the control group, whose gloves were only changed prior to cementation [36].

During sterile procedures, it is important that surgical staff wear double, high-quality, sterile gloves and closely monitor the integrity of the gloves throughout the entirety of the procedures. Additionally, regularly changing the surgeon's outer gloves may improve sterility and decrease the likelihood of perforation.

3.5   Anesthesia and Blood Conservation/Tranexamic Acid

General anesthesia and neuraxial anesthesia (epidural or spinal anesthesia) are common anesthetic practices performed during total hip and total knee arthroplasties. The techniques utilized are typically based on surgeon and the anesthesia team's preference. Anesthesia can have significant systemic effects on the patient's organ systems during and after the surgical procedure, potentially inhibiting the immune system's ability to ward off infection [37]; therefore, anesthesia should always be considered a risk factor for SSI.

Chang et al. performed a retrospective study of 3081 patients who received total hip and total knee arthroplasties over 4 years. Via multivariate regression analysis and propensity score matching, they compared the rates of SSI in patients who received either general anesthesia or neuraxial anesthesia. This study found that patients who received general anesthesia during total hip or total knee arthroplasties had a significantly greater risk of SSI within 30 days of surgery ($p = 0.002$) [38]. Additionally, a meta-analysis of 13 studies was performed and found neuraxial anesthesia, compared to general anesthesia, was associated with a significant reduction in postoperative SSI in patients receiving total hip or knee arthroplasties [39].

The current evidence suggests that neuraxial anesthesia is superior to general anesthesia at preventing SSIs in patients undergoing total hip and knee arthroplasty surgeries [37, 38] and should be the anesthesia of choice, if possible. Along with choosing the correct anesthesia technique, managing adequate blood volume may be beneficial to prevent SSIs. Significant intraoperative blood loss may lead to postoperative anemia, increasing the likelihood of the patient needing allogenic blood transfusions. Unfortunately, to date, there has yet to be any study demonstrating a direct link between allogenic blood transfusions and SSI in patient status-post TJA; however, it is believed that allogenic blood transfusions have an immune-modulating effect, which could decrease the immune system's ability to perform its normal protective functions, thus increasing the risk of infection [40].

Tranexamic acid (TXA) is commonly used in the realm of orthopedic surgery to prevent intraoperative blood loss, especially during TJA, where blood loss can be significant. TXA is a synthetic lysine analogue, which blocks the lysine binding sites on plasminogen. This mechanism gives TXA its marked anti-fibrinolytic properties, which have been shown to prevent the breakdown of clots [41]. In 2010, a randomized, placebo-controlled trial was performed assessing the effects of TXA on death, vascular occlusive events, and blood transfusions in over 20,000 trauma patients in 274 hospitals, across 40 countries [42]. This study was impressive in that it found a significant reduction in all-cause mortality from bleeding compared to placebo ($p = 0.0035$). They noted a significant decrease in the amount of blood transfusions required after TXA, and there was no difference in vascular occlusive events between groups. Although this study was focused on patients who fell victim to trauma, it parallels patients undergoing significant surgical procedures, such as TJA. Recent evidence has supported its use in reducing the rate of PJI after TJA as well. A retrospective study by Yazdi et al. on over 6000 patients who underwent primary TJA found that the administration of TXA significantly reduced the rate of PJI after TJA, even after controlling for known confounding variables via multivariate regression analysis [43].

TXA can be administered intravenously, orally, or topically. Current guidelines suggest all routes of administration are superior to placebo and are all equal at preventing blood loss [44]. It is strongly recommended that TXA is utilized during TJA procedures to minimize significant intraoperative blood loss and prevent the need for allogenic blood transfusions.

3.6 Intraoperative Measures (Irrigation Solutions, Antimicrobial Powder, Operative Time)

3.6.1 Irrigation Solutions

Utilizing solutions to irrigate surgical sites in an attempt to prevent SSI is common practice among surgeons of all subspecialties, with 97% of surgeons reporting using intraoperative irrigation [45]. Flowing solution over a wound can help rid the surgical site of harmful bacteria and residual debris. There are multiple options for irrigation solutions, including povidone-iodine (Betadine), normal saline, and antibiotics. Based on the current evidence, the CDC currently suggests the use of aqueous povidone-iodine solution over saline solution as the ideal agent to irrigate incisional wounds for the purposes of preventing SSI during clean and clean-contaminated procedures [46]. Antibiotic solutions have also been utilized to potentially decrease the risk of SSI. However, a meta-analysis consisting of five randomized controlled trials found no significant difference between antibiotic irrigation, normal saline, and no irrigation [44].

It is important to be mindful that the current evidence is considered low-quality and would benefit from well-designed randomized controlled trials to definitively identify the ideal irrigation agent and procedures for preventing SSI.

3.6.2 Antimicrobial Powder

Antimicrobial powders have been used by surgeons to provide large, local bactericidal effects to the surgical incision site with minimal systemic exposure to the patient. One of the most widely utilized antimicrobial powders is intrawound vancomycin [47]. Vancomycin has bactericidal effects, particularly against gram-positive bacteria, by way of inhibiting bacterial cell wall synthesis [48]. Currently, there is conflicting evidence on the efficacy of using intrawound vancomycin powder to prevent SSI and PJI. Bakhsheshian et al. performed a systematic review of 18 studies that compared intrawound vancomycin during spine surgery versus their standard practice without intrawound vancomycin [45]. Their results concluded vancomycin powder is effective at decreasing SSI in spine surgery patients; however, the majority of the studies in this review were class III level evidence, with only one randomized controlled trial included in the analysis. The use of vancomycin powder during TJA procedures has yet to be well established. One study performed a retrospective cohort analysis, which noted a significant decrease in rates of PJI after using intrawound vancomycin powder, but only in TJA revisions, not in primary TJA procedures [49].

On a systemic level, vancomycin typically has relatively only mild adverse reactions including tissue irritation, phlebitis, fevers, chills, and, on a rare occasion, ototoxicity. However, nephrotoxicity is a known and potentially devastating complication; therefore, administering additional medications with known nephrotoxic effects should be avoided, and systemic drug levels should be routinely monitored [46]. Johnson et al. performed a study on 34 TJA patients who received 2 g of intrawound vancomycin powder and assessed serum and wound vancomycin levels over 24 hours. The study found that vancomycin levels in the wound remained at highly therapeutic levels averaging greater than 900 µg/mL at 3 hours and greater than 200 µg/mL at 24 hours. They extrapolate, with an intrawound vancomycin half-life of 7.2 hours, it would take approximately 64 hours for wound levels to fall below the 2 µg/mL minimal therapeutic level to inhibit *S. aureus*. Additionally, serum levels remained well below the minimal therapeutic dose [50]. Although this study demonstrated minimal systemic exposure with intrawound vancomycin, they did not assess vancomycin's ability to prevent PJI.

In conclusion, outside of spinal surgery, the use of vancomycin powder in the prevention of PJI cannot be recommended due to the current conflicting data and poor-quality studies.

3.6.3 Operative Time

Multiple studies have demonstrated and increased cumulative risk of intraoperative infection associated with prolonged operative time. Extended surgical times may result in increased foot traffic from OR staff and increased exposure of the surgical site and sterile operative equipment to potentially harmful airborne pathogens, among other secondary consequences. Increased OR foot traffic leads to an increased number of door openings which has been shown to increase the rates of SSI and can also potentially disrupt the efficacy of laminar air flow systems [25, 27].

It is also important to consider the effects of prolonged operative times on personal protective equipment (PPE), as well as the sterile operative equipment. Glove perforation is a well-known complication of surgical procedures. The incidence of glove perforation and contamination has been shown to increase with prolonged surgical times [51, 52]. One study showed a significant reduction in glove perforation when changing the surgeon's gloves in 20-minute intervals [35]. Additional evidence shows an increased rate of glove perforations after 90 minutes [34, 53]; therefore, surgeons are encouraged to routinely exchange outer gloves within this timeframe. Despite the promising literature in regard to preventing glove perforation, to date, there is no data to suggest that changing an intact, impermeable, surgical gown will reduce the incidence of SSI, regardless of operative time. Finally, it is imperative to minimize the amount of time sterile trays are open by keeping them closed until they are specifically needed for that procedure. A study by Dalstrom et al. noted a direct correlation between the duration of open, uncovered, exposure of sterile trays to increased contamination [54]. Additionally, they found that simply covering the open trays with a sterile towel could significantly decrease the contamination risk [52].

3.7 Antibiotic Prophylaxis

3.7.1 Current Recommendation

Prophylactic antibiotics are a critical modality that should be utilized in all surgical patients to prevent SSIs. In accordance with the CDC, the American Academy of Orthopaedic Surgeons (AAOS) recommends, in the absence of allergies, first- or second-generation cephalosporins (cefazolin and cefuroxime) to be administered via single intravenous dose within 60 minutes prior to surgical incisions (Table 3.1) [55]. Additionally, they recommend terminating antibiotics postoperatively within 24 hours. The antibiotic chosen to be administered during surgery should be effective at specifically eliminating the most common infectious pathogens. These pathogens include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Proteus* [56]. It is imperative to refrain from using broad-spectrum antibiotics, if possible, to avoid creating resistant escape mutants. Exposing patients to

Table 3.1 American Academy of Orthopaedic Surgeons' recommendations for prophylactic antibiotics [53]

• Utilize cefazolin or cefuroxime, if there are no allergies present
• If allergy exists, vancomycin and clindamycin are recommended for prophylaxis
• Receive prophylactic antibiotics within 1 hour prior to surgical incision
• Vancomycin should be administered 2 hours prior to surgical incision, and administered again between 6 and 12 hours if necessary
• Have prophylactic antibiotics discontinued within 24 hours following surgery

Table 3.2 Dosing recommendations [10]

Cefazolin	• Patient weight > 60–80 kg = 2.0 g
	• Patient weight > 120 kg = 3.0 g
Vancomycin	• 15 mg/kg IV
Clindamycin	• 600–800 mg
	• Klk

vancomycin may be linked to be an increase in outbreaks of *vancomycin-resistant Enterococcus* (*VRE*) and *methicillin-resistant Staphylococcus aureus* (*MRSA*) [54]. However, in cases of known cephalosporin allergies, vancomycin and clindamycin are recommended. Additionally, vancomycin can be used in patients who are known carriers of MRSA or are at high risk of MRSA infections, such as those living in a nursing home or healthcare workers. Due to the prolonged infusion time, vancomycin is recommended to be administered 2 hours before incision, and for extended surgical operations, again in 6–12 hours [57].

Proper antibiotic dosing is critical for effective prophylaxis. An adequate dose is required to maintain the minimum inhibitory concentration (MIC) throughout the entirety of procedure to prevent the growth of harmful pathogens. Failure to maintain MIC has been found to increase the risk of wound infection [58]. See Table 3.2 for specific dosing recommendations.

3.7.2   Routes of Administration

Intravenous (IV) administration has been considered to be the ideal route of administration for maintaining the minimum inhibitory concentration (MIC) [10]. The infusions of antibiotics should be administered 30–60 minutes prior to incision time, and MIC should be maintained throughout the procedure. Additional routes such as intraosseous administration have been studied. However, high-quality evidence to support its efficacy is lacking and should be further explored. Thus far, one study on 2293 patients who underwent spinal and arthroplasty surgeries and received irrigation solution with vancomycin and IV polymyxin found no readmission for primary joint infections [59]. Additional high-level evidence is still lacking, and, due to the absence of strong evidence to the contrary, IV administration remains the most effective to deliver antibiotics in surgical practice.

3.8 Antimicrobial Resistance

The use of antimicrobial drugs has saved countless lives from infections that were once considered life-threatening since their widespread inception in the early twentieth century after Sir Alexander Fleming discovered modern-day penicillin. Despite their effectiveness at ridding sick patients of various infectious organisms, their extensive utilization has resulted in the inevitable creation, selection, and survival of drug-resistant organisms across all medical specialties throughout the world. Although microbes have an innate ability to mutate to form resistance, the recent widespread resistance can be contributed mostly to the improper use of antibiotics. For example, prescribing antibiotics to patients with upper respiratory symptoms consistent with a viral, not bacterial, pathogen is all but common in practice. Additionally, the prolonged use of broad-spectrum antimicrobials is believed to have contributed to drug resistance organisms as well. In 2010, the World Health Organization (WHO) estimated that roughly 23,000 patients die every year related to infections from resistant organisms [46].

Unfortunately, the research and production of effective antimicrobial agents has been declining over the past few decades, despite the increased need [60]. The large number of existing antimicrobials and push for medical providers to limit the administration of these drugs have significantly limited drug sales. In fact, several large pharmaceutical companies no longer perform infection prevention research or produce antimicrobials altogether due to lack of profits [58]. To combat the current crisis involving drug-resistant organisms, proper use of antimicrobials by the prescribing healthcare professional is paramount. Providers should be always intimately familiar with the drugs they are prescribing. It is important, if plausible, to identify the pathogen associated with the infections and utilize the specific antimicrobial drug that can neutralize the infection in the quickest and safest way possible. Prolonged use of broad-spectrum antimicrobials should be avoided unless clinically necessary. Also, prescribing certain drugs simply because the patient requests them could result in undesired outcomes.

3.9 Wound Dressings and Topical Antimicrobial Products

3.9.1 Occlusive vs. Silver Impregnated vs. Dry Gauze

Proper wound dressing is a critical step in minimizing postoperative SSI and PJI. Surgical wounds from TJI can be considered unique from other surgeries in that they can present with significant drainage [61], requiring a dressing that is highly absorptive. Additionally, TJA surgical incision sites are located over joint spaces and therefore should be durable enough to withstand the forces from the early mobility that is likely to occur with rehabilitation, but also pliable enough to allow for changes in joint edema [59]. There are multiple dressings available including

occlusive dressings; gauze impregnated with antimicrobial agents, such as silver; and simple dry gauze. Dry gauze simply provides a protective layer over the surgical wound and provides no additional antimicrobial properties or moistening effects. Dry gauze may have favorable fluid handling capacity, which can decrease the frequency of dressing changes and maceration; however, they do not facilitate quicker wound healing by maintaining a moist environment. Occlusive dressings provide airtight and water-resistant protection of the surgical site via an outer wax coating; however, they do not have the fluid handling capacity of dry gauze. A recent systematic review found there are significantly fewer wound complications when using occlusive dressings compared to dry gauze [59]. Dressings have been impregnated with metals such as silver for its bactericidal effects. The rationale being that the silver ions will disrupt any local bacteria growth in the surgical wound and further prevent SSI. Unfortunately, there have yet to be any high-quality studies that demonstrate a significant decrease in SSIs when using silver-impregnated gauze compared to standard dressings. Therefore, the additional cost of these advanced dressings cannot be justified.

3.9.2 Antimicrobial-Coated Sutures

Triclosan is a broad-spectrum antimicrobial agent that has been used in many medical and consumer products since the 1960s [62]. In the last two decades, triclosan-coated sutures have been utilized to further prevent SSIs. To date, triclosan-coated sutures have been shown to decrease SSI in numerous surgical disciplines. Unfortunately, there are few studies specific to orthopedic surgery. One randomized controlled trial conducted by Sprowson et al. compared rates of SSI when using triclosan-coated sutures vs. standard sutures in patients who received elective total hip arthroplasty (THA) and total knee arthroplasty (TKA) [63]. While the results of this study found there was no difference in SSI rates between the two groups, the study had a number of limitations including the use of a quasi-randomized selection technique and lack of control for surgeon skill and incision type. In contrast, a meta-analysis of multiple surgical disciplines showed a significant decrease in SSIs when using triclosan-coated sutures when compared to standard sutures [64]. Due to an abundance of high-quality evidence across multiple surgical disciplines that demonstrates the positive antimicrobial effects of triclosan-coated sutures, its use is recommended as an additional measure to reduce the risk of SSIs.

3.9.3 Vacuum-Assisted Dressings

Vacuum-assisted dressings, also known as negative pressure wound therapy (NPWT), have been utilized across several surgical specialties for decades. These dressings utilize suction over the wound to create a negative pressure environment.

The negative pressure is thought to accelerate the wound healing process by removing excess exudate, increasing granulation tissue, promoting angiogenesis, and causing wound contraction [65, 66]. There does appear to be some benefit to using vacuum-assisted dressings for patients who are considered high risk for developing infections, for example, in cases of orthopedic trauma. Stannard et al. performed a prospective randomized controlled trial to investigate NPWT's ability to prevent wound dehiscence and infections in high-risk orthopedic trauma patients. They found a decrease in infection rates and wound dehiscence in patients who had NPWT applied to their surgical wound after closure [67]. Another study found a reduction in infection rates in patients who suffered open tibial fractures [68].

Despite promising evidence for high-risk patients, the use of vacuum-assisted dressings in low-risk cases has not been shown to reduce rates of deep infections, reoperation, and wound dehiscence [69, 70]. Therefore, the prophylactic use of NPWT in low-risk, uncomplicated cases cannot be recommended over standard dressings due to the lack of efficacy and increased cost burden.

3.9.4   Topical Incisional Sealants

Topical incisional sealants, such as Integuseal, Dermabond, etc., are popular among many surgical disciplines. The attractiveness of topical sealants stems from its ability to easily create a barrier over the surgical incision site allowing for little to no use of suturing material or staples and, in theory, reducing rates of infection and wound drainage. Despite the promising rationale behind topical sealants, a recent randomized controlled trial found no difference in scar outcome and infections rates after using topical sealants vs. staples in patients who underwent THA procedures [71]. Two additional randomized controlled trials also found there is no difference in the rates of surgical site infections when using topical sealants in patients who received THA and TKA [72, 73]. In fact, utilizing topical sealants over the joints with high tensile forces, such as the knee, may be inappropriate due to increased rates of wound dehiscence compared to sutures and staples [74].

Due to the lack of data supporting the use of topical incisional sealants in orthopedic surgical cases, it is not recommended as an effective adjunct to prevent surgical site infections.

3.9.5   Biofilm Mapping: Detection and Localization

The complex structures consisting of both cellular and acellular components, formed via the symbiotic cooperation of microorganisms on surfaces such as total joint prosthesis, are known as a biofilm. Specifically, bacterial biofilms contain an aggregation of pathogens surrounded by a network of extracellular matrix that aids in creating a physical defense against the body's natural immune system [75].

Biofilm formation on various surfaces has been thoroughly documented in the past [76, 77]. It is also known that mature biofilms have limited permeability to neutrophils [10]. These biofilms can generate critical problems for orthopedic surgeons performing total joint arthroplasties, due to their difficulty to detect, prevent, and treat. Biofilm mapping is an attempt to identify and localize biofilms on various surfaces. One study was able to successfully grow and identify *Pseudomonas aeruginosa* biofilm on 316 L stainless steel orthopedic screws in a lab setting using confocal microscopy. The mapping noted patchy film deposits on the shaft and within the threads of the screws; however, there was no specific pattern to the deposition of the film [78]. Unfortunately, *Pseudomonas aeruginosa* strains are not typically encountered on orthopedic prostheses, in contrast to strains of *Staphylococcus epidermidis* and *Staphylococcus aureus.*

Additionally, Kobayashi et al. examined the utility of ultrasonication and real-time polymerase chain reaction (RT-PCR) to detect *Staphylococcus aureus* biofilm [79]. They found an exposure of ultrasound between 1 and 5 minutes to the structural surface disrupted the biofilm and allowed for its detection. Additionally, they found ultrasonication exposure greater than 5 minutes lysed the bacterial cells making them unable to be detected. However, there are limitations to this study. The biofilm was only detected on stainless steel surfaces, so the results cannot be extrapolated to the various structural surfaces on encountered in total joint arthroplasties.

Although there is some promising literature on the topic of biofilm mapping, the evidence is primarily based on the laboratory setting. To date there are no high-quality studies that have demonstrated a practical approach to identifying the detection and location of biofilms in the clinical setting. Thus, the clinical relevance of biofilm mapping is unknown at this time.

3.10 Biomaterials, Carriers, Coatings, and Novel Technologies

3.10.1 *Material Composition of Orthopedic Components*

In the realm of orthopedic surgery, there are a host of components with varying material compositions including bone cement, titanium, and stainless steel. The different material compositions could allow for varying degrees of biofilm deposition on their surfaces. In rabbit models, Sheehan et al. found an increased ability of *Staphylococcus epidermidis* species to adhere biofilms to stainless steel surfaces of femoral intramedullary components compared to titanium surfaces by a rate of nearly 150% [80]. One theory is that stainless steel has a higher surface free energy (> 40 mN/m) compared to titanium, thus allowing for increased pathogen binding. They also examined silver-coated implants and found there to be no difference in the development of biofilms when compared to control metals. The main limitation of this study was that the animal model does not directly correlate to clinical

practice. An additional study examined the ability of *Staphylococcal* species (spp.) to adhere to varying structural surfaces which included bone cement, stainless steel, and titanium [81]. They noted bone cement to have a significantly higher rate of bacterial biofilm adherence ($p < 0.05$) followed by stainless steel and then titanium. Also, Lauderdale et al. found *Staphylococcus aureus* biofilm on titanium to be increasingly susceptible to rifampin and levofloxacin treatment [82], which further indicates that titanium may currently be the ideal metal of choice for orthopedic components.

Research has demonstrated particular material properties that increase biofilm production including porosity, hydrophobicity/hydrophilicity, and roughness. Put simply, bacteria can form biofilm on essentially any component [10]. As a result, to date there is no material composition that is known to be immune to biofilm growth [10].

3.10.2   Intrinsically Bioactive Materials

Bioactive materials have been used for decades due to their known intrinsic antimicrobial effects. One of the most commonly used metal coatings is silver. Rather recently, silver nanoparticles have been used as coatings on orthopedic implants due to their significant bactericidal effects against both gram-positive and gram-negative organisms [83]. The mechanism of action behind silver's bactericidal effects is believed to be secondary to disruption of the bacterial cell membrane as well as the inhibition of bacterial enzymes, thus making it an ideal coating substance for preventing peripheral joint infections [81]. One study found a decrease in biofilm formation on the surface of silver-coated implants with antimicrobial potency which was positively correlated to the concentration of silver coating [84]. Although the antimicrobial effects of silver-coated prosthesis appear to have a promising future, it is possible, albeit rare, for resistant bacterial strains to develop [85].

3.10.3   Bioactive Antibacterial Coatings
and Surface Modification

Implant-related infections are both costly and potentially fatal postoperative complications associated with TJA. Surface modifications such as nanotubes within orthopedic implants and antibacterial coatings are currently being evaluated to improve antibiotic capabilities and prevent biofilm adhesion on implant surfaces in patients undergoing TJA. One study performed in a sheep model evaluated the efficacy of vancomycin-coated orthopedic prosthesis in its ability to inhibit biofilm formation [86]. The results of the study demonstrated an inhibition of biofilm formation from strains of *Staphylococcus aureus* as well as increased osseointegration,

compared to controls with no antibiotic coating. Other implant coatings, such as iodine, have also been a topic of interest in clinical research. A clinical trial was performed and examined the use of iodine-coated implants in the prevention and treatment of postoperative infections in 222 patients following TJA [87]. The results of the study found that all implant-associated infections were prevented or cured by the 18-month follow-up. There were also no reported cytotoxic or adverse effects [85]. Additional research supports the antibacterial efficacy of iodine coatings on surfaces of insertion pins for the purposes of external fixation [88].

Silver has long been known to have excellent antimicrobial abilities with low cytotoxicity toward the host [89, 90]. Slane et al. performed a study to assess the antibiofilm properties of commercial bone cement loaded with silver nanoparticles. Although the cement did not have any antimicrobial effects against planktonic bacteria, there was a significant reduction in the formation of biofilm [91]. Further supporting evidence demonstrated silver nanoparticles significantly reduced biofilm formation from strains of *Pseudomonas aeruginosa and Staphylococcus epidermidis* [92].

Additionally, an implant coating known as human ß-3 defensin has shown promising antibiofilm effects. Human ß-3 defensin is a 45-amino acid peptide that is a subclass of mammalian defensins that can be found in human bone tissue and bone cells [93]. Huang et al. performed a study and reported human ß-3 defensin to have significant antibiofilm effects against methicillin-resistant *Staphylococcus aureus* (MRSA) compared to clindamycin and vancomycin [91]. An additional study noted human ß-3 defensin was also effective at inhibiting biofilm formation from strains of methicillin-resistant *Staphylococcus epidermidis* (MRSE) [94].

3.10.4   Antibiotic Carriers

Antibiotic carriers, such as antibiotic-loaded cements and beads, have been used to provide locally delivered antimicrobial effects in patients with PJI. The infections caused from biofilm-forming bacteria on the surfaces of orthopedic implants are notoriously difficult to eliminate due to the innate antibiotic resistance provided by the matrix of the film [95]. Calcium sulfate and calcium phosphate are two commonly used antibiotic carrier compounds currently being used. An in vitro study found that gentamicin-loaded beads containing calcium sulfate were able to prevent and eradicate biofilm of gram-positive bacteria [96]. Although this is an in vitro study, this may demonstrate some clinical relevance to using gentamicin-loaded beads to eliminate biofilm-related infections. Also, Stravinskas et al. reported local antibiotic levels, after using a single dose, to be between 100 and 1000 times the minimal inhibitory concentration within the first few days of treatment, which lasted up to 4 weeks in elderly patients with chronic osteomyelitis [97]. On the contrary, a long-term retrospective study was performed that compared the efficacy of debridement vs. debridement plus calcium-sulfate pellets in 65 patients with adult chronic osteomyelitis [98]. This study found there was no difference in the healing rates

between the two groups. Additionally, a study was performed to examine the use of calcium sulfate beads in 33 patients who were undergoing irrigation and debridement procedures secondary to total hip and knee arthroplasty infections [99]. The author did not find significant improvements in outcomes when utilizing the calcium sulfate beads in addition to the irrigation debridement. Therefore, the author did not recommend their use due to lack of efficacy and increased costs associated.

Overall, there is a current lack of consistent high-quality evidence to suggest the use of antibiotic carriers to locally manage PJIs. Providers are encouraged to use their clinical judgment when discerning whether to incorporate antibiotic carriers into their treatment plan.

3.11 Novel Technologies

PJIs continue to be one of the most common causes of total hip and knee arthroplasty revisions [100]. These patients are typically subject to multiple surgical revision procedures, prolonged hospital stays, and increased healthcare costs [101]. On a systemic level, healthcare costs secondary to PJI are estimated to be $771 million per year, as of 2011 [102]. Despite the devastating effects of PJI on patients and the healthcare system alike, a "gold standard" test for accurately diagnosing PJIs remains unidentified. Future technologies are currently being researched to improve our PJI diagnostic abilities. These technologies include interleukin-6 (IL-6), serum D-dimer, synovial alpha-defensin, and next-generation sequencing (NGS).

Interleukin-6 (IL-6) is a peptide released from the body's immune cells, known as a cytokine. This cytokine can be released in response to injury and inflammation as an acute phase reactant [103]. In addition to inflammation, IL-6 can also induce plasma cell development and stimulate osteoclastic activity [104]. Due to the increase in serum IL-6 levels during an inflammatory reaction, it is proposed that this can be a potential marker to diagnose PJI. In 2010, a meta-analysis was performed to assess the sensitivity and specificity of specific biomarkers in PJI diagnosis, namely, C-reactive protein (CRP) and IL-6 [101]. The results of the study found IL-6 to be the most accurate with a sensitivity of 0.97 (95% CI 0.93–0.99) and a specificity of 0.91 (95% CI 0.87–0.94). Second to IL-6 was CRP with a sensitivity of 0.88 (95% CI 0.86–0.90) and a specificity of 0.74 (95% CI 0.71–0.76). However, on the contrary, a prospective study by Randau et al. demonstrated IL-6 to have sensitivity ranging from 0.49 to 0.79 and a specificity ranging from 0.58 to 0.88 [105]. Unfortunately, due to the lack of consistent evidence supporting the accuracy of IL-6 and the increased cost associated with measuring serum and synovial IL-6 levels, this biomarker has not been widely accepted as a diagnostic tool in clinical practice.

Additional diagnostic technologies include next-generation sequencing (NGS), which is a collection of genetic sequencing techniques that can be used to identify pathogens quickly, compared to traditional cultures [106]. NGS searches genomic databases containing specific genetic information for various pathogens and matches

this data with the pathogen in question. NGS is typically utilized via two methods, shotgun metagenomics and 16S amplicon. Shotgun metagenomics involves sequencing all of the DNA in a sample and identifying which organism the DNA came from [107]. This method was studied by Thoendel et al., and found they were able to detect 43.9% of pathogens in PJI patients who were previously culture-negative [105]. 16S amplicon uses polymerase chain reaction (PCR) to amplify 16S ribosomal DNA which is specific to the pathogen. One study was able to demonstrate clinical utility of 16S amplicon by successfully identifying *Streptococcus canis* from a patient who had been previously diagnosed with a culture-negative PJI [108]. Two additional studies were also able to successfully detect pathogens in culture-negative PJIs [109, 110]. Although there may be future clinical utility for NGS, additional high-level data collection is required to further validate and refine NGS techniques for consistently accurate PJI diagnoses.

D-dimers are protein products that become elevated in the serum when plasmin causes the degradation of fibrin clots. Serum D-dimer levels can become elevated from multiple pathologies including venous thromboembolism, recent surgery, increased age, and pregnancy [111]. In addition, a prospective study was performed demonstrating elevated serum D-dimer to have a sensitivity of 0.89 and a specificity of 0.93, which was found to be superior at diagnosing PJI in 245 primary and revision arthroplasty patients, compared to ESR and CRP [112]. Obtaining serum D-dimer levels is both cost-effective and readily accessible in the clinical setting. However, more research is required to confirm its use as a PJI diagnostic test.

Alpha-defensin is a microbicidal mammalian defensin peptide that is released from activated neutrophils in the presence of infection and is active against many gram-positive and gram-negative bacteria, enveloped viruses, and fungi [113]. Alpha-defensins act by binding to the pathogen's cell membrane and creating pores within the membrane, thus increasing cellular permeability and destruction [114]. Due to the apparent specificity to infections, serum alpha-defensin levels have been studied to attempt to use this biomarker as a tool to diagnose PJI in patients. Currently, there are two methods of to measure serum alpha-defensin levels, which include the alpha-defensin immunoassay and the lateral flow assay technique. A systematic review was performed to evaluate the diagnostic accuracy of the lateral flow technique compared to the immunoassay technique [115]. The immunoassay technique was identified as being the superior diagnostic test when compared to the lateral flow technique. Their respective sensitivities were found to be 0.96 (95% CI 0.90–0.98) vs. 0.71 (95% CI 0.55–0.83). There was no statistically significant difference in their specificities at 0.96 (95% CI 0.93–0.97) and 0.90 (95% CI 0.81–0.95), respectively; thus, both techniques could potentially be used to accurately rule in PJI in patients [113]. Additional studies have found similar results in regard to the immunoassay's superior diagnostic capabilities compared to the lateral flow technique, although both tests are extremely specific for PJI [116, 117]. Due to the high specificity of both tests, measuring alpha-defensin levels would be an ideal test for diagnosing PJI. However, there are negative aspects to these tests. The cost of a single alpha-defensin test was estimated to be approximately $760 [118], and the immunoassay technique requires the samples to be shipped off to a specialized lab

for testing, although results are obtained within 24 hours [114]. The lateral flow technique is cheaper and can be performed in-house typically within 20 minutes. However some studies show that lab results may be dependent on the skill of the lab technician [114, 116].

3.11.1 Nanotubes

Nanotubes are hollow, cylindrical structures with diameters typically from 1 to 800 nm. These nanotubes can be incorporated into orthopedic implants, which provide clinical advantages such as delivering local antimicrobial effects and improved osseointegration [119]. Research has demonstrated a reduced blood flow around the area of prosthesis, therefore potentially impeding a local immune response should an infection arise [120]. The utilization of antibiotic-loaded nanotubes could combat infections from within the implant and could potentially decrease the need for traditional systemic antibiotic treatment. Titanium dioxide nanotubes within orthopedic implants are produced via surface modification and are inherently antibacterial with the capability of successfully loading antibacterial drugs [121]. Li et al. performed an in vitro study to assess the antibacterial effects of zinc-loaded titanium nanotubules compared to titanium nanotubules. The results of the study showed zinc-coated titanium to have a significant reduction in bacterial growth compared to titanium nanotubules alone. Additionally, nanotube diameters between 70 and 100 nm have been found to increase osteoblast differentiation, therefore improving osseous integration [122]. Zinc is required for osteoblastic activity, alkaline phosphate (ALP) activity, and collagen synthesis [123]. Li et al. also noted zinc-loaded titanium nanotubules to have increased ALP activity. Another supporting study by Popat et al. examined *Staphylococcus epidermidis* adhesion on the surface of gentamicin-loaded titanium nanotubes [124]. The results of the study indicate loading nanotubes with gentamicin will significantly reduce initial surface adhesions from *S. epidermidis*. Additionally, they reported increased osteoblastic activity in nanotubes with and without gentamicin.

Although the antimicrobial effectiveness of nanotubes appears promising, due to the current lack of high-level evidence, the clinical applicability of nanotubule technology remains unclear. Further research in animal and human subjects is required.

3.11.2 Bacteriophages

Bacteriophages are natural viruses that can target specific bacteria strains to inject their DNA and lyse the prokaryotic bacterial cell. After bacterial cell lysing, the newly produced phages are released into the extracellular space where they again target remaining bacterial cells. The specificity of the bacteriophages allows for adequate antibacterial effects without compromising host eukaryotic cells and gut

flora. One recent clinical trial evaluated bacteriophage therapy in the treatment of *Staphylococcus aureus* in two patients who had refractory peripheral joint infections [125]. They performed a salvage procedure which consisted of debridement, antibiotics, and implant retention. In addition to the salvage procedure, the patient was injected locally with a bacteriophage mix. The results of this trial were significant in that both patients had favorable clinical outcomes at follow-up and had no adverse reactions. Additionally, a double-blinded, randomized controlled trial, conducted by Wright et al., used an injectable mix of six bacteriophages specific to targeting antibiotic-resistant *Pseudomonas aeruginosa*, in an attempt to treat chronic otitis media infections [126]. The authors were able to successfully treat the chronic infections, indicating a possible role for phage therapy in antibiotic-resistant infections.

Overall, phage therapy appears to be a promising treatment option for eliminating the bacteria associated with peripheral joint infections. Still, additional research is needed to determine appropriate treatment parameters, such as route of administration, dose, duration, and timing.

3.11.3   Vaccines

The use of vaccinations to immunize patients against common strains of infectious bacteria is currently a popular topic in the field of orthopedics. The basis for prophylactically vaccinating patients prior to elective orthopedic procedures would be to prevent infections and biofilm formation. This would also help prevent the need for antibiotic medications that could potentially create resistant strains. One of the most commonly researched pathogen vaccines is for *Staphylococcus aureus* as it is frequently associated with peripheral joint infections [127]. Several in vitro studies have been performed, and they demonstrated the effectiveness of vaccinations preventing the formation of biofilm. One study investigated the use of recombinant *S. aureus* binding proteins as potential vaccine antigens [128]. The study found a significant reduction in *S. aureus* surface adhesion when preexposed to anti-surface binding protein antigens.

One of the most advanced vaccinations currently in development is the four-antigen *S. aureus* vaccine (S4Ag). The vaccine targets key virulence factors that are necessary for *S. aureus* to initiate and maintain infection [129]. A double-blinded, placebo-controlled phase 2 clinical trial, known as the *STaphylococcus aureus* suRgical Inpatient Vaccine Efficacy (STRIVE) study, was performed to assess the safety and efficacy of S4Ag vaccination in adults undergoing elective open posterior spinal fusion procedures with multilevel instrumentation. Subjects received either a placebo injection or the S4Ag vaccine 10–60 days prior to surgical intervention and were monitored for infection up until 180 days after surgery. The results of the study indicated that the S4Ag vaccine may be a safe and efficacious method to preventing *S. aureus* infections in elective orthopedic procedures. Currently, the STRIVE study remains in phase 3 clinical trials.

Unfortunately, there are currently no vaccinations that have been approved for orthopedic use by the Food and Drug Administration (FDA). However, prophylactic immunization against infectious pathogens may be a promising method for preventing surgical site infections should future high-quality trials support their clinical use.

3.11.4 Bioactive Enzymes

Bioactive enzymes are proteins that can be used to eliminate the biofilm matrix that forms on the surfaces of orthopedic implants. These proteins include proteases, deoxyribonucleases, and glycosidases [130]. One advantage of using bioactive enzymes is that, compared to antimicrobials, there is a reduction in the risk of creating resistant strains of bacteria [131]. A biofilm-degrading glycoside hydrolase, known as dispersin B (DsP), has been found to have some antibiofilm effects. One study reported that when coating DsP on surfaces, there was over a 98% decrease in biofilm production in two strains of *S. epidermidis* [132]. It is believed that DsP is effective against biofilms because of its ability to cleave poly-N-acetylglucosamine (PNAG). PNAG is a component of the three-dimensional extracellular matrix of biofilm. Additionally, Kaplan et al. studied the effects of recombinant human DNase I (rhDNase) on biofilm inhibition [133]. They reported that rhDNase significantly inhibited the formation of biofilm from *Staphylococcus epidermidis* and *Staphylococcus aureus*. Also, the study noted rhDNase was able to detach preformed biofilm produced by *Staphylococcus aureus*.

3.11.5 Shockwave Treatment, Electromagnetic Fields, and Electrical Stimulation

Bacterial biofilm adhesion on orthopedic implants creates a significant risk for developing PJI. Bacteria within the biofilm have an increased resistance to antibiotic medications, creating infections that are notoriously difficult to manage. Nonpharmaceutical modalities have been researched to assist traditional antibiotics in biofilm eradication. One in vitro study investigated the effect of pulsed electromagnetic field (PEMF) on the efficacy of antibiotics in the treatment of infection of implants [134]. *Staphylococcus epidermidis* biofilm was grown for 5 days on stainless steel surgical screws. Two groups were established in the study. The experimental group was exposed to PEMF, as well as treatment with gentamicin. The control group only received treatment with gentamicin, without PEMF exposure. The results of the study showed a significant reduction of biofilm of at least 50% in the experimental group compared to the control group. This study demonstrates that the use of PEMF may be an effective modality to disrupt biofilm architecture, allowing for improved antibiotic infiltration.

Laser-generated shockwave treatment is an additional biofilm eradicating method that has been researched. A study by Kizhner et al. evaluated the effects of laser-generated shockwaves to interrupt biofilm formation on common metallic and plastic medical devices [135]. The results of the study demonstrated a 98% reduction in *Pseudomonas aeruginosa* biofilm formation when applying laser-generated shockwaves for 4–10 seconds [133]. 24-hour ultrasound-generated shockwaves were also examined in an in vivo study and were found to be effective at eliminating *Escherichia coli* biofilms, when combined with gentamicin treatment [136]. Cathodic-voltage-controlled electrical stimulation (CVCES) was recently evaluated in a study by Canty et al. [137]. The study found that CVCES alone, at −1.8 V, completely eradicated biofilm-associated colony-forming units (CFU) for both *P. aeruginosa* and MRSA. This study shows potentially promising future applications of CVEC for the eradication of bacterial biofilms.

3.12 Conclusion

Despite the abundance of literature and promising novel technologies on the horizon, periprosthetic joint infections continue to be a devastating healthcare burden and impair patient quality of life. This chapter discussed many of the relevant clinical topics in the field of infection prevention. Although progress is being made, further collaboration between clinicians and scientists is necessary to continue progressing toward better prevention and management of musculoskeletal infections.

References

1. Whitehouse JD, Friedman ND, Kirkland KB, Richardson WJ, Sexton DJ. The Impact of Surgical-Site Infections Following Orthopedic Surgery at a Community Hospital and a University Hospital Adverse Quality of Life, Excess Length of Stay, and Extra Cost. Infection Control & Hospital Epidemiology. 2002;23(04):183–189.
2. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic Burden of Periprosthetic Joint Infection in the United States. *J Arthroplasty*. 2012;27(8):61–65.e1.
3. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351:1645–54.
4. Kapadia BH, McElroy MJ, Issa K, et al. The economic impact of periprosthetic infections following total knee arthroplasty at a specialized tertiary-care center. J Arthroplasty 2014;29(5):929.
5. Jiranek W, Kigera JWM, Klatt BA, et al. General Assembly, Prevention, Host Risk Mitigation - General Factors: Proceedings of International Consensus on Orthopedic Infections. J Arthroplasty. October 2018.
6. Alamanda VK, Springer BD. Perioperative and Modifiable Risk Factors for Periprosthetic Joint Infections (PJI) and Recommended Guidelines. *Curr Rev Musculoskelet Med*. 2018;11(3):325–331.
7. Sørensen LT, Hemmingsen U, Kallehave F, et al. Risk factors for tissue and wound complications in gastrointestinal surgery. Ann Surg. 2005;241(4):654–658.

8. Leijh PC, Nathan CF, van den Barselaar MT, van Furth R. Relationship between extracellular stimulation of intracellular killing and oxygen-dependent microbicidal systems of monocytes. Infect Immun. 1985;47:502–507.
9. Riber U, Espersen F, Skinhoj P, et al. Induction of oxidative burst response in human neutrophils by adherent staphylococci. Comparison between Staphylococcus epidermidis and Staphylococcus aureus.APMIS1993; 101:55–60.
10. Sørensen LT. Wound healing and infection in surgery: the clinical impact of smoking and smoking cessation: a systematic review and meta-analysis. Arch Surg. 2012;147(4):373–383.
11. International Consensus Group. *Proceedings of the Second International Consensus Meeting (ICM) on Musculoskeletal Infection*. Data Trance Publishing Company; 2018.
12. Boraiah S, Joo L, Inneh IA, Rathod P, Meftah M, Band P, & Iorio R (2015). Management of modifiable risk factors prior to primary hip and knee arthroplasty. Journal of Bone and Joint Surgery American, 97(23), 1921–1928.
13. Mangram AJ, et al. Guideline for prevention of surgical site infection, 1999. Am J Infect Control. 1999;27(2):97–134.
14. Xiao G, Chen Z, Lv X. Chlorhexidine-based body washing for colonization and infection of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*: an updated meta-analysis. Infect Drug Resist. 2018;11:1473–1481.
15. Kamel C, McGahan L, Polisina J, Miezwinski-Urban M,Embil JM. Preoperative skin antiseptic preparations for preventing surgical site infections: A systematic review. Infection Control and Hospital Epidemiology 2012;33:608–17.
16. Chlebicki MP, Safdar N, O'Horo JC, Maki DG: Preoperative chlorhexidine shower or bath for prevention of surgical site infection: A meta-analysis. Am J Infect Control 2013;41(2):167–173.
17. Colling K, Statz C, Glover J, Banton K, Beilman G. Pre-operative antiseptic shower and bath policy decreases the rate of S. aureus and methicillin-resistant S. aureus surgical site infections in patients undergoing joint arthroplasty. Surg Infect. 2015;16(2):124–132.
18. Kapadia BH, Johnson AJ, Issa K, Mont MA. Economic evaluation of chlorhexidine cloths on healthcare costs due to surgical site infections following total knee arthroplasty. J Arthroplasty 2013;28:1061–5.
19. Lefebvre A, Saliou P, Lucet JC, Mimoz O, Keita-Perse O, Grandbastien B, et al. Preoperative hair removal and surgical site infections: network meta-analysis of randomized controlled trials. J Hosp Infect 2015;91:100–8.
20. Tanner J, Norrie P, Melen K. Preoperative hair removal to reduce surgical site infection. Cochrane Database Syst Rev 2011:CD004122.
21. Seropian R., Reynolds, B.M. Wound infections after preoperative depilatory versus razor preparation. Am J Surg, 121 (1971), pp. 251–254.
22. Balthazar ER, Colt JD, Nichols RL. Preoperative hair removal: a random prospective study of shaving versus clipping. South Med J 1982;75:799–801.
23. Daines BK, Dennis DA, Amann S. Infection prevention in total knee arthroplasty. J Am Acad Orthop Surg 2015;23:356–64.
24. Cacciari P, Giannoni R, Marcelli E, et al. Cost evaluation of a ventilation system for operating theatre: an ultraclean design versus a conventional one. Ann Ig. 2004;16:803–809.
25. Whyte W., Hodgson, R., J. Tinkler. The importance of airborne bacterial contamination of wounds. J Hosp Infect, 3 (1982), pp. 123–135.
26. Salvati EA, Robinson RP, Zeno SM, et al. Infection rates after 3175 total hip and total knee replacements performed with and without a horizontal unidirectional filtered air-flow system. J Bone Joint Surg Am. 1982;64:525.
27. Brandt, C, Hott, U, Sohr D, et al. Operating room ventilation with laminar airflow shows no protective effect on the surgical site infection rate in orthopedic and abdominal surgery" Annals of Surgery, vol. 248, no. 5, pp. 695–700, 2008.
28. Young R., O'Regan D. Cardiac surgical theatre traffic: Time for traffic calming measures? Interact. Cardiovasc. Thorac. Surg. 2010;10:526–529.

29. Ritter MA. Operating room environment. Clin Orthop Relat Res. 1999;369:103–9.

30. Bedard M., Pelletier-Roy R., Angers-Goulet M., Leblanc P. A., & Pelet S. (2015). Traffic in the operating room during joint replacement is a multidisciplinary problem. Canadian Journal of Surgery, 58(4), 232–236.

31. Ward WG, Sr., Cooper JM, Lippert D, Kablawi RO, Neiberg RH, Sherertz RJ. Glove and gown effects on intraoperative bacterial contamination. Annals of surgery. 2014;259: 591–7.

32. Blom AW, Gozzard C, Heal J, Bowker K, Estela CM. Bacterial strike-through of reusable surgical drapes: the effect of different wetting agents. Journal of Hospital Infection. 2002;52:52–55.

33. Garibaldi, RA, Maglio, S, Lerer, T, Becker, D, Lyons, R. Comparison of nonwoven and woven gown and drape fabric to prevent intraoperative wound contamination and postoperative infection. *Am J Surg* 1986;152(5:505–9.

34. Maffuli N, Capasso G, Testa V. Glove perforation in elective orthopaedic surgery. Acta Orthop Scand 1989;60:565–6.

35. Misteli H, Weber WP, Reck S, Rosenthal R, Zwahlen M, Fueglistaler P, et al. Surgical glove perforation and the risk of surgical site infection. Arch Surg. 2009;144(6):553–558.

36. Al-Maiyah M, Bajwa A, Mackenney P, Port A, Gregg PJ, Hill D, et al. Glove perforation and contamination in primary total hip arthroplasty. The Journal of bone and joint surgery British volume. 2005;87: 556–9.

37. Kettner SC, Willschke H, Marhofer P. Does regional anaesthesia really improve outcome? Br J Anaesth. 2011;107(Suppl 1):90–5.

38. Chang CC, Lin HC, Lin HW, Lin HC. Anesthetic management and surgical site infections in total hip or knee replacement: a population-based study. Anesthesiology. 2010;113(2):279–84.

39. Zorrilla-Vaca A, Grant MC, Mathur V, Li J, Wu CL. The impact of neuraxial versus general anesthesia on the incidence of postoperative surgical site infections following knee or hip arthroplasty: a meta-analysis. Reg Anesth Pain Med. 2016;41:555–563.

40. Viola J, Gomez MM, Restrepo C, Maltenfort MG, Parvizi J. Preoperative anemia increases postoperative complications and mortality following total joint arthroplasty. J Arthroplasty 2015;30:846–8.

41. De-jie, Fu; Cheng, Chen; Lin, Guo; Liu, Yang. In Chinese Journal of Traumatology. April 2013 16(2):67–76.

42. Shakur H, Roberts I, Bautista R, et al. Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. Lancet 2010;376(9734):23–32.

43. Yazdi H, Klement MR, Hammad M, et al. Tranexamic Acid Is Associated With Reduced Periprosthetic Joint Infection After Primary Total Joint Arthroplasty. The Journal of Arthroplasty. 2019.

44. Fillingham YA, Jevsevar DS, Yates AJ, Sayeed SA, Sah AP, Bini SA, et al. Tranexamic Acid in Total Joint Arthroplasty: The Clinical Practice Guides of the American Association of Hip and Knee Surgeons, American Academy of Orthopaedic Surgeons, Hip Society, Knee Society, American Society of Regional Anesthesia and Pain Medicine. 2017.

45. Whiteside OJ, Tytherleigh MG, Thrush S, Farouk R, Galland RB. Intra-operative peritoneal lavage – who does it and why? Ann R Coll Surg Engl. 2005;87(4):255–8.

46. Global Guidelines for the Prevention of Surgical Site Infection. Geneva: World Health Organization; 2016.

47. J. Bakhsheshian, N.S. Dahdaleh, S.K. Lam, J.W. Savage, Z.A. Smith. The use of vancomycin powder in modern spine surgery: systematic review and meta-analysis of the clinical evidence. World Neurosurg, 83 (2015), pp. 816–823.

48. Katzung BG, Trevor AJ. Basic and Clinical Pharmacology. 14th ed. New York; McGraw-Hill Education; 2018.

49. Otte J.E., Politi J.R., Chambers B., Smith C.A. Intrawound vancomycin powder reduces early prosthetic joint infections in revision hip and knee surgery. Surg Technol Int. 2017;30:284–289.

50. J.D. Johnson, J.M. Nessler, R.D. Horazdovsky, S. Vang, A.J. Thomas, S.B. Marston. Serum and wound vancomycin levels after intrawound administration in primary total joint arthroplasty. J Arthroplasty, 32 (2017), pp. 924–928.
51. Bukhari SS, Harrison RA, Sanderson PJ. Contamination of surgeons' gloves fingertips during surgical operations. J Hosp Infect 1993;24:117–21.
52. Hollaus PH, Lax F, Janakiev D. Glove perforation rate in open lung surgery. Euro J Cardiothorac Surg 1999;15:461–4.
53. Hübner NO, Goerdt AM, Stanislawski N, Assadian O, Heidecke CD, Kramer A, et al. Bacterial migration through punctured surgical gloves under real surgical conditions. BMC Infect Dis 2010;10:192.
54. Dalstrom DJ, Venkatarayappa I, Manternach AL, Palcic MS, Heyse BA, Prayson MJ. Time-dependent contamination of opened sterile operating-room trays. The Journal of bone and joint surgery American volume. 2008;90: 1022–5.
55. American Academy of Orthopaedic. American Academy of Orthopaedic Surgeons. Recommendations for the use of intravenous antibiotic prophylaxis in primary total joint arthroplasty. Information Statement 1027. 2011.
56. Illingworth KD, Mihalko WM, Parvizi J, Sculco T, McArthur B, El Bitar Y, et al. How to minimize infection and thereby maximize patient outcomes in total joint arthroplasty: A multicenter approach. Journal of Bone and Joint Surgery – Series A 2013;95.
57. Meehan J, Jamali AA, Nguyen H. Prophylactic Antibiotics in Hip and Knee Arthroplasty. The Journal of Bone and Joint Surgery-American Volume 2009;91:2480–90.
58. Forse RA, Karam B, MacLean LD, Christou NV. Antibiotic prophylaxis for surgery in morbidly obese patients. Surgery. 1989;106:750–756. discussion 756–757.
59. Whiteside LA. Prophylactic peri-operative local antibiotic irrigation. The Bone & Joint Journal 2016;98-B:23–6.
60. Spellberg B, Guidos R, Gilbert D, et al. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis. 2008;46:155–164.
61. Sharma G, Lee SW, Atanacio O, Parvizi J, Kim TK: In search of the optimal wound dressing material following total hip and knee arthroplasty: A systematic review and meta-analysis. Int Orthop 2017;41:1295–1305.
62. Jones RD, Jampani HB, Newman JL, Lee AS. Triclosan: a review of effectiveness and safety in health care settings. Am J Infect Control 2000;28:184–96.
63. Sprowson AP, Jensen C, Parsons N, Partington P, Emmerson K, Carluke I, et al. The effect of triclosan-coated sutures on the rate of surgical site infection after hip and knee arthroplasty: a double-blind randomized controlled trial of 2546 patients. The Bone & Joint Journal 2018;100-B:296–302.
64. De Jonge SW, Atema JJ, Solomkin JS et al (2017) Meta-analysis and trial sequential analysis of triclosan-coated sutures for the prevention of surgical-site infection. Br J Surg 104:e118–e133.
65. Siqueira MB, Ramanathan D, Klika AK, Higuera CA, Barsoum WK. Role of negative pressure wound therapy in total hip and knee arthroplasty. World J Orthop 2016;7:30–7.
66. Moues CM, van den Bemd GJ, Heule F, Hovius SE. Comparing conventional gauze therapy to vacuum-assisted closure wound therapy: a prospective randomised trial. J Plast Reconstr Aesthet Surg 2007;60:672e81.
67. Stannard JP, Volgas DA, McGwin G, Stewart RL, Obremskey W, Moore T, et al. Incisional negative pressure wound therapy after high-risk lower extremity fractures. J Orthop Trauma 2012;26:37–42.
68. Stannard JP, Volgas DA, Stewart R, McGwin G, Alonso JE. Negative pressure wound therapy after severe open fractures: a prospective randomized study. J Orthop Trauma 2009;23:552–7.
69. Cooper HJ, Bas MA. Closed-Incision Negative-Pressure Therapy Versus Antimicrobial Dressings After Revision Hip and Knee Surgery: A Comparative Study. J Arthroplasty 2016;31:1047–52.

70. Helito CP, Bueno DK, Giglio PN, Bonadio MB, Pécora JR, Demange MK. Negative-Pressure Wound Therapy In The Treatment Of Complex Injuries After Total Knee Arthroplasty. Acta Ortop Bras 2017;25:85–8.
71. Glennie RA, Korczak A, Naudie DD, Bryant DM, Howard JL. MONOCRYL and DERMABOND vs Staples in Total Hip Arthroplasty Performed Through a Lateral Skin Incision: A Randomized Controlled Trial Using a Patient-Centered Assessment Tool. J Arthroplasty 2017;32:2431–5.
72. Khan RJK, Fick D, Yao F, Tang K, Hurworth M, Nivbrant B, et al. A comparison of three methods of wound closure following arthroplasty: a prospective, randomised, controlled trial. J Bone Joint Surg Br 2006;88:238–42.
73. Siddiqui M, Bidaye A, Baird E, Abu-Rajab R, Stark A, Jones B, et al. Wound dressing following primary total hip arthroplasty: a prospective randomised controlled trial. J Wound Care 2016;25:40, 42–5.
74. Coulthard P, Esposito M, Worthington HV, van der Elst M, van Wae OJF, Darcey J. Tissue adhesives for closure of surgical incisions. Cochrane Database Syst Rev 2010:CD004287.
75. Bazaka K., Jacob M. V., Crawford R. J., Ivanova E. P. Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. *Applied Microbiology and Biotechnology*. 2012;95(2):299–311.
76. Dunne WM Jr. Bacterial adhesion: seen any good biofilms lately? Clin Microbiol Rev. 2002;15:155–166.
77. Habash M, Reid G. Microbial biofilms: their development and significance for medical device-related infections. J Clin Pharmacol. 1999;39:887–898.
78. P. Stoodley, S. Kathju, F. Z. Hu et al., "Molecular and imaging techniques for bacterial biofilms in joint arthroplasty infections," Clinical Orthopaedics and Related Research, 437, 31–40, 2005.
79. Kobayashi N, Bauer TW, Tuohy MJ, Fujishiro T, Procop GW. Brief ultrasonication improves detection of biofilm-formative bacteria around a metal implant. Clin Orthop Relat Res 2007;457:210–3.
80. Sheehan E, McKenna J, Mulhall KJ, Marks P, McCormack D. Adhesion of Staphylococcus to orthopaedic metals, an in vivo study. Journal of Orthopaedic Research 2004;22:39–43.
81. Gad GFM, Aziz AAA, Ibrahem RA. In-vitro adhesion of *Staphylococcus* spp. to certain orthopedic biomaterials and expression of adhesion genes. J Appl Pharm Sci (2012) 2(6):145–9.
82. Lauderdale KJ, Malone CL, Boles BR, Morcuende J, Horswill AR. 2010. Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. J Orthop Res 28:55–61.
83. Rai, M.K.; Deshmukh, S.D.; Ingle, A.P.; Gade, A.K. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. J. Appl. Microbiol. 2012, 112, 841–852.
84. Harrasser, N. et al. Antibacterial efficacy of titanium-containing alloy with silver-nanoparticles enriched diamond-like carbon coatings. AMB Express 5, 1 (2015).
85. Hobman, J.L.; Crossman, L.C. Bacterial antimicrobial metal ion resistance. J. Med. Microbiol. 2014, 64, 471–497.
86. Stewart S, Barr S, Engiles J, Hickok NJ, Shapiro IM, Richardson DW, et al. Vancomycin-modified implant surface inhibits biofilm formation and supports bone-healing in an infected osteotomy model in sheep: a proof-of-concept study. J Bone Joint Surg Am. 2012;94:1406–15.
87. Tsuchiya H, Shirai T, Nishida H, Murakami H, Kabata T, Yamamoto N, et al. Innovative antimicrobial coating of titanium implants with iodine. J Orthop Sci. 2012;17:595–604.
88. Shirai T, Watanabe K, Matsubara H, Nomura I, Fujiwara H, Arai Y, et al. Prevention of pin tract infection with iodine-supported titanium pins. J Orthop Sci. 2014;19:598–602.
89. Hardes J, Ahrens H, Gebert C, Streitbuerger A, Buerger H, Erren M, et al. Lack of toxicological side-effects in silver-coated megaprostheses in humans. Biomaterials 2007;28:2869–75.
90. Gosheger G, Hardes J, Ahrens H, Streitburger A, Buerger H, Erren M, et al. Silver-coated megaendoprostheses in a rabbit model--an analysis of the infection rate and toxicological side effects. Biomaterials 2004;25:5547–56.

91. Slane J, Vivanco J, Rose W, Ploeg H-L, Squire M. Mechanical, material, and antimicrobial properties of acrylic bone cement impregnated with silver nanoparticles. Mater Sci Eng C Mater Biol Appl. 2015;48:188–96.

92. Kalishwaralal K, BarathManiKanth S, Pandian SRK, Deepak V, Gurunathan S. Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis. Colloids Surf B Biointerfaces. 2010;79:340–4.

93. Huang Q, Yu H-J, Liu G-D, Huang X-K, Zhang L-Y, Zhou Y-G, et al. Comparison of the effects of human β-defensin 3, vancomycin, and clindamycin on Staphylococcus aureus biofilm formation. Orthopedics. 2012;35:e53–60.

94. Zhu C, Tan H, Cheng T, Shen H, Shao J, Guo Y, et al. Human β-defensin 3 inhibits antibiotic-resistant Staphylococcus biofilm formation. J Surg Res. 2013;183:204–13.

95. W. Zimmerli, P. Sendi, Orthopaedic biofilm infections, APMIS: Acta Pathol. Microbiol. Immunol. Scand. 125 (2017) 353–364.

96. Butini ME, Cabric S, Trampuz A, Di Luca M. In vitro anti-biofilm activity of a biphasic gentamicin-loaded calcium sulfate/hydroxyapatite bone graft substitute. Colloids and Surfaces B: Biointerfaces 2018;161:252–260.

97. Stravinskas M, Horstmann P, Ferguson J. Pharmacokinetics of gentamicin eluted from a regenerating bone graft substitute: in vitro and clinical release studies. Bone Joint Res. 2016;5(09):427–435.

98. Chang W, Colangeli M, Colangeli S, Di Bella C, Gozzi E, Donati D. Adult osteomyelitis: debridement versus debridement plus Osteoset T pellets. Acta Orthopaedica Belgica 2007;73:238–43.

99. Flierl MA, Culp BM, Okroj KT, Springer BD, Levine BR, Della Valle CJ. Poor Outcomes of Irrigation and Debridement in Acute Periprosthetic Joint Infection With Antibiotic-Impregnated Calcium Sulfate Beads. J Arthroplasty 2017;32:2505–7.

100. Bozic KJ, SM, Lau E, et al. The Epidemiology of Revision Total Knee Arthroplasty in the United States. *Clin Orthop Relat Res*. 2010;468(1):45–51.

101. Bozic KJ. The Impact of Infection After Total Hip Arthroplasty on Hospital and Surgeon Resource Utilization. *J Bone Jt Surg Am*. 2005;87(8):1746.

102. Kurtz SM, Ong KL, Lau E, Bozic KJ, Berry D, Parvizi J. Prosthetic joint infection risk after TKA in the medicare population. Clin Orthop Relat Res. 2010;468(1):52–56. doi:https://doi.org/10.1007/s11999-009-1013-5.

103. Berbari E, Mabry T, Tsaras G, et al. Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systematic review and meta-analysis. *J Bone Jt Surg Am*. 2010;92(11):2102–2109.

104. Song M, Kellum JA. Interleukin-6: *Crit Care Med*. 2005;33(Suppl):S463-S465.

105. Randau TM, Friedrich MJ, Wimmer MD, et al. Interleukin-6 in serum and in synovial fluid enhances the differentiation between periprosthetic joint infection and aseptic loosening. *PLoS One*. 2014;9(2):e89045.

106. Dunne WM, Westblade LF, Ford B. Next-generation and whole-genome sequencing in the diagnostic clinical microbiology laboratory. *Eur J Clin Microbiol Infect Dis*. 2012;31(8):1719–1726.

107. Thoendel M, Jeraldo P, Greenwood-Quaintance KE, et al. A Novel Prosthetic Joint Infection Pathogen, Mycoplasma salivarium, Identified by Metagenomic Shotgun Sequencing. *Clin Infect Dis*. 2017;65(2):332–335.

108. Tarabichi M, Alvand A, Shohat N, Goswami K, Parvizi J. Diagnosis of Streptococcus canis periprosthetic joint infection: the utility of next-generation sequencing. *Arthroplasty Today*. 2017;4(1):20–23.

109. Tarabichi M, Shohat N, Goswami K, et al. Diagnosis of Periprosthetic Joint Infection: The Potential of Next-Generation Sequencing. *J Bone Jt Surg*. 2018;100(2):147–154.

110. Tarabichi M, Shohat N, Goswami K, Parvizi J. Can next generation sequencing play a role in detecting pathogens in synovial fluid? *Bone Jt J*. 2018;100-B(2):127–133.

111. Kabrhel C, Mark Courtney D, Camargo CA Jr., Plewa MC, Nordenholz KE, Moore CL, et al. Factors associated with positive D-dimer results in patients evaluated for pulmonary embolism. Acad Emerg Med 2010,17:589–597.

112. Shahi A, Kheir MM, Tarabichi M, Hosseinzadeh HRS, Tan TL, Parvizi J. Serum D-Dimer Test Is Promising for the Diagnosis of Periprosthetic Joint Infection and Timing of Reimplantation. *J Bone Jt Surg Am*. 2017;99(17):1419–1427.

113. Selsted ME, White SH, Wimley WC (1995). "Structure, function, and membrane integration of defensins". Curr. Opin. Struct. Biol. 5 (4): 521–527.

114. Lehrer RI, Barton A, Daher KA, Harwig SS, Ganz T, Selsted ME. Interaction of human defensins with Escherichia coli. Mechanism of bactericidal activity. *J Clin Invest*. 1989;84(2):553–561.

115. Eriksson HK, Nordstrom J, Gabrysch K, Hailer NP, Lazarinis S. Does the Alpha-defensin Immunoassay or the Lateral Flow Test Have Better Diagnostic Value for Periprosthetic Joint Infection? A Systematic Review. *Clin Orthop Relat Res*. 2018;476(5):1065–1072.

116. Suen K, Keeka M, Ailabouni R, Tran P. Synovasure "quick test" is not as accurate as the laboratory-based alpha-defensin immunoassay: a systematic review and meta-analysis. *Bone Jt J*. 2018;100-B(1):66–72.

117. Wyatt MC, Beswick AD, Kunutsor SK, Wilson MJ, Whitehouse MR, Blom AW. The Alpha-Defensin Immunoassay and Leukocyte Esterase Colorimetric Strip Test for the Diagnosis of Periprosthetic Infection: A Systematic Review and Meta-Analysis. *J Bone Jt Surg Am*. 2016;98(12):992–1000.

118. Alvand A, Rezapoor M, Parvizi J. The Role of Biomarkers for the Diagnosis of Implant-Related Infections in Orthopaedics and Trauma. In: Drago L, ed. A Modern Approach to Biofilm-Related Orthopaedic Implant Infections. Vol 971. Cham: Springer International Publishing; 2017:69–79.

119. Ganguly DY, Shahbazian R, Shokuhfar T. Recent advances in nanotubes for orthopedic implants. J Nanotech Smart Mater. 2014;1:1–10.

120. Pulido L, Ghanem E, Joshi A, Purtill JJ, Parvizi J 2008. Periprosthetic joint infection: the incidence, timing, and predisposing factors. Clin Orthop Relat.466:1710-5

121. Li Y, Xiong W, Zhang C, Gao B, Guan H, Cheng H, et al. Enhanced osseointegration and antibacterial action of zinc-loaded titania-nanotube-coated titanium substrates: in vitro and in vivo studies. J Biomed Mater Res A 2014;102:3939–50.

122. Wang N, Li HY, Lu WL, Li JH, Wang JS, Zhang ZT, Liu Y. Effects of TiO2 nanotubes with different diameters on gene expression and osseointegration of implants in minipigs. Biomaterials 2011;32:6900–6911.

123. Palacios C. The role of nutrients in bone health, from A to Z. CritRev Food Sci Nutr 2006;46:621–628.

124. Popat KC, Eltgroth M, LaTempa TJ, Grimes CA, Desai TA. Decreased Staphylococcus epidermis adhesion and increased osteoblast functionality on antibiotic-loaded titania nanotubes. Biomaterials 2007;28:4880–4888.

125. Ferry T., Leboucher G., Fevre C., Herry Y., Conrad A., Josse J., Batailler C., Chidiac C., Medina M., Lustig S., et al. Salvage Debridement, Antibiotics and Implant Retention ("DAIR") With Local Injection of a Selected Cocktail of Bacteriophages: Is It an Option for an Elderly Patient With Relapsing Staphylococcus aureus Prosthetic-Joint Infection? Open Forum. Infect. Dis. 5;2018:ofy269.

126. Wright A, Hawkins CH, Änggård EE, Harper DR. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clinical Otolaryngology 2009;34:349–57.

127. Gustin M-P, Giard M, Bénet T, Vanhems P. Use of surveillance data to identify target populations for Staphylococcus aureus vaccines and prevent surgical site infections: a pilot study. Hum Vaccin Immunother 2014;10:3517–21.

128. Ratcliffe E. Staphylococcus aureus Binding Proteins for Prevention of Orthopaedic Implant-Related Infections. Journal of Microbial & Biochemical Technology 2014;6:303–13.

129. Gurtman A, Begier E, Mohamed N, Baber J, Sabharwal C, Haupt RM, Edwards H, Cooper D, Jansen KU, Anderson, AS. The development of a staphylococcus aureus four antigen vaccine for use prior to elective orthopedic surgery. Hum Vaccin Immunother 2019;15(2)358–370.
130. Kaplan J.B. Biofilm dispersal: Mechanisms, clinical implications, and potential therapeutic uses. J. Dent. Res. 2010;89:205–218.
131. Hancock R.E., Patrzykat A. Clinical development of cationic antimicrobial peptides: From natural to novel antibiotics. Curr. Drug Targets Infect. Disord. 2002;2:79–83.
132. Pavlukhina S.V., Kaplan J.B., Li X., Wei C., Yu X., Madhyastha S. Noneluting enzymatic antibiofilm coatings. Appl Mater Interfaces. 2011;4(9):4708–4716.
133. Kaplan JB, LoVetri K, Cardona ST, Madhyastha S, Sadovskaya I, Jabbouri S, Izano EA. 2012. Recombinant human DNase I decreases biofilm and increases antimicrobial susceptibility in staphylococci. J Antibiot (Tokyo) 65:73–77.
134. Pickering SAW, Bayston R, Scammell BE. Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants. J Bone Joint Surg Br. 2003;85:588–93.
135. Kizhner V, Krespi YP, Hall-Stoodley L, Stoodley P. Laser-generated shockwave for clearing medical device biofilms. Photomed Laser Surg. 2011;29:277–82.
136. Rediske AM, Roeder BL, Brown MK, Nelson JL, Robison RL, Draper DO, et al. Ultrasonic enhancement of antibiotic action on Escherichia coli biofilms: an in vivo model. Antimicrob Agents Chemother. 1999;43:1211–4.
137. Canty MK, Hansen LA, Tobias M, Spencer S, Henry T, Luke-Marshall NR, Campagnari AA, Ehrensberger MT. 2019. Antibiotics enhance prevention and eradication efficacy of cathodic-voltage controlled electrical stimulation against titanium-associated methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa biofilms. mSphere 4:e00178–19.

Chapter 4
Prosthetic Infection: Colonization and Diagnosis

Mark Wu and Thorsten M. Seyler

Abstract Joint replacement procedures improve quality of life, function, and mobility, for over a million individuals annually. With the aging population, along with increased rates of diagnosis and treatment of arthritis, the number of these procedures continues to rise. Prosthetic joint infections (PJIs) are one of the most serious complications of joint replacement surgery both from a patient and healthcare perspective. Those with PJI report poor satisfaction with their surgery and have an overall lower health-related quality of life. There is also an immense cost to the healthcare system. PJI costs in the USA exceeded $900 million in 2012 and have been projected to exceed $1.6 billion over the following decade. The heavy burden of these complications has sparked interest in gaining a better understanding of the mechanism, diagnosis, prevention, and treatment of PJI. This chapter covers the topics of defining and classifying a prosthetic infection and biofilm formation as well as describes the tests and tools used for diagnosing a PJI.

Keywords Prosthetic infection · Biofilm · Diagnosis · Morbidity · Pathogenesis · Colonization · Resistance

4.1 What Is a Prosthetic Joint Infection (PJI) and How Is It Classified?

4.1.1 Implant Use in the USA

Joint replacement procedures improve quality of life, function, and mobility, for over a million individuals annually [1, 2]. With the aging population, along with increased rates of diagnosis and treatment of arthritis, the number of these procedures continues to rise. In 2010, the prevalence of patients living with a total hip replacement was 2.5 million and 4.7 million living with a total knee replacement [3]. Another study projected that the need for total hip arthroplasty (THA) would

M. Wu · T. M. Seyler (✉)
Division of Adult Reconstruction, Department of Orthopaedic Surgery, Duke University, Durham, NC, USA
e-mail: thorsten.seyler@dm.duke.edu

reach over 500,000, and over 3 million for total knee arthroplasty (TKA) by 2030 [4]. In addition to knee and hip arthroplasty, many patients now undergo shoulder, elbow, and ankle arthroplasty as well [5]. Prosthetic joint infections (PJIs) are one of the most serious complications of joint replacement surgery both from a patient and healthcare perspective.

4.1.2 Morbidity and Mortality of Prosthetic Joint Infection (PJI)

PJI is an infection involving the joint prosthesis and adjacent tissue [5]. The incidence of PJI after primary hip or knee arthroplasty ranges from 0.5% to 2.5% [5–8] and accounts for up to 25% of the revision surgeries performed [9, 10]. PJI contributes significantly to patient morbidity and mortality [6]. Prior studies have reported up to 25.8% rate of all-cause mortality within 2 years and as high as 45% at 5 years and 50% mortality rate for those with recurrent infections [11–13]. Additionally, those with PJI report poor satisfaction with their surgery and have an overall lower health-related quality of life [13]. There is also an immense cost to the healthcare system. PJI costs in the USA exceeded $900 million in 2012 and have been projected to exceed $1.6 billion over the following decade [6]. The heavy burden of these complications has sparked interest in gaining a better understanding of the mechanism, diagnosis, prevention, and treatment of PJI. This primary focus chapter will focus on understanding how PJI is classified, the role of biofilm in infection, and how to clinically diagnose PJI, along with advances in this area.

4.1.3 Classification of PJI

There are multiple classification schemes that have been proposed, though the clinical classification used most frequently is based on the timing of infection since surgery [14–18]. These categories are acute, chronic/delayed, and late/acute hematogenous. The distinction between categories can differ between studies. For instance, some literature defines an acute infection as less than a month from the procedure, while others define it as less than 3 months [16, 17]. In practice, most clinicians and literature will consider acute PJI as infection occurring within 3 months. Delayed or chronic PJI occurs after 3 months, but before 12–24 months. Finally, late-onset infection will occur after 12 or 24 months and is most frequently caused by hematogenous infection [17]. However, it may also be due to very indolent infection initiated at the time of the procedure [5]. Classifying PJI based on time of onset is useful, because it provides insight into the potential causative organism(s) and clinical management. Acute infections are generally due to virulent pathogens, such as Gram-negative bacilli, streptococci, and *Staphylococcus aureus*,

acquired at the index procedure or due to wound dehiscence [19]. Delayed or chronic PJI is often due to less virulent organisms such as coagulase-negative staphylococci or *Cutibacterium* species acquired at the time of index surgery [19, 20].

Another classification scheme described by Tsukayama in the 1990s categorized PJI into four different categories [16]. The first category had positive intraoperative cultures in the setting of suspected initial suspected aseptic loosening. The second category was early postoperative infection that developed less than a month after the procedure. Late-chronic infection was one that developed 1 month or more after the index procedure, and the last category was defined as acute hematogenous infection, associated with a documented or suspected antecedent bacteremia [16]. Additional classification schemes exist incorporating factors such as host type, microorganism, clinical presentation, and other factors [21, 22]. In 2002, McPherson and colleagues proposed a classification system for PJI (Table 4.1) that classifies infection type as early postoperative (<4 weeks), acute hematogenous (< 4-week duration), and late-chronic infection (>4-week duration. In addition to timing of infection, it also classifies the host into uncompromised, compromised, or significant compromise based on different factors depicted in Table 4.1.

Lastly, it grades the extremity based on the number of compromising factors present. In this germinal paper, the authors reported significant correlations with a patient's stage of disease and whether they were more likely to die or have their legs amputated (i.e., those with later stage disease had worse prognosis) [22]. Another classification that has been used to stage prosthetic joint infections is the Cierny and DiPasquale classification for osteomyelitis. In their study, they prospectively staged patients with PJI according to a previously described classification system for

Table 4.1 McPherson classification for prosthetic joint infection. (Adapted from McPherson et al. Clin Orthop Relat Res. 2002 [22])

Infection Type	Systemic Host Grade	Local Extremity Grade
I: Early postoperative infection (<4 weeks post-operative) II: Hematogenous infection (<4 weeks duration) III: Late chronic infection (>4 weeks duration)	A: Uncompromised B: Compromised (1-2 compromising factors) C: Significant compromise (>2 compromising factors) or one of the following: - Absolute neutrophil count <1000 - CD4 T cell count <100 - IV drug abuse - Chronic active infection at another site - Dysplasia or neoplasm of the immune system	1: Uncompromised 2: Compromised (1-2 compromising factors) 3: Significant compromise (>2 compromising factors)
Systemic Host Grade Compromising factors	Age>80, immunosuppressive drugs, alcoholism, malignancy, chronic active dermatitis or cellulitis, pulmonary insufficiency, chronic indwelling catheter, renal failure requiring dialysis, chronic malnutrition, systemic inflammatory disease, current nicotine use, systemic immune compromise, diabetes, hepatic insufficiency	
Local Extremity Grade compromising factors	Active infection present >3-4 months, multiple incision with skin brideges, soft tissue loss from prior trauma, subcutaneous abscess >8cm^2, synovial cutaneous fistula, prior periarticular fracture or trauma about a joint, prior local irradiation, vascular insufficiency	

osteomyelitis in adult patients [23]. Specifically, PJIs were entered as anatomic types of the disease: early and superficial osteomyelitis (Type II) or late and refractory osteomyelitis (Type IV of the initial osteomyelitis staging system). Cierny et al. also described local and systemic host factors that affect treatment and prognosis (Table 4.2) that were used to stage patients. Both the host classification (A, healthy; B, compromised by one or more local and/or systemic parameters; or C, hosts have morbidity that surpasses their capacity to withstand curative treatment) and anatomic type of disease (Type II or IV) were combined to direct the selection of patient for surgery. They reported that all of the treatment failures, deaths, and amputations occurred in high-risk patients that were prospectively identified according to their staging system [24]. Both of these studies emphasized the importance of infection duration and the condition of the patient (host) in determining patient prognosis and guiding treatment.

4.2 PJI Pathogenesis, the "Golden Period," and the Role of Biofilm

4.2.1 Pathogenesis of Infection

The majority of PJI occur through inoculation of microorganisms intraoperatively, either through direct contact or aerosolized contamination of the prosthesis [5, 17]. As previously mentioned, more virulent microorganisms will typically cause earlier manifestation of PJI. After inoculation, the microorganism will adhere to the prosthesis and/or periprosthetic tissue. Studies have demonstrated that a much lower inoculum of bacteria is needed to initiate infection in the presence of a prosthesis due to the formation of a biofilm [25]. Another mechanism of colonization is through direct spread of infection. This can occur if a nearby infection such as cellulitis or osteomyelitis spreads into the joint. An open periprosthetic fracture resulting in direct contact with the outer world would be another possible mechanism of inoculation [20].

Table 4.2 Local and systemic host factors affect treatment and prognosis adapted from Cierny and DiPasquale. Clin Orthop Relat Res. 2002 [24]

B(L)-host (local compromise)	B(S)-host (systemic compromise)
Chronic lymphedema	Malnutrition
Venous stasis	Immune deficiencies
Major vessel disease	Chronic hypoxia
Arteritis	Malignancies
Extensive scarring	Diabetes mellitus
Radiation fibrosis	Extremes of age (<2 years, >70 years
Retained foreign bodies	Chronic tobacco abuse (>40 pack years)
(suture, buckshot)	Current tobacco abuse
	Major organ failure

   The third mechanism of infection is hematogenous seeding of the prosthesis from a distant primary focus. While susceptible to hematogenous seeding throughout their lifetime, literature suggests that implants are more susceptible in the first years postoperatively possibly due to the increased vascularity about the implant during this time period [5, 26]. The reported frequency of hematogenous seeding of prostheses is varied in the literature, and also varies depending on the infecting microorganism. One study reported a 30–40% rate of PJI in *S. aureus* bacteremia [27]. A recent report investigated microbiological patterns in prosthetic joint infections [28]. In 926 patients who developed 997 PJIs, 35% were classified as hematogenous infectious. Ninety-nine percent of these infections were monomicrobial, with *S. aureus* (28%) being the most frequently isolated species. Overall, streptococci species (39%), most commonly group B streptococci, and staphylococci species (36%) were the most commonly isolated. Gram-negative rods (12%) were the third most common group. Only 1% of hematogenous PJI were polymicrobial. Skin, teeth, and gastrointestinal tract infections were the most common primary sites of infection [28]. These findings are overall consistent with prior reports [29–31]. Table 4.3 demonstrates the common species causing PJI.

4.2.2   The "Golden Period"

When microorganisms first contact with the prosthesis intraoperatively, they immediately adhere to the implant surface and begin the process of forming a biofilm. Studies have demonstrated that prevalence of infection during these initial hours depends on the number of bacteria present and the immune status of the host. In the first 2 hours, the host defenses will decrease the overall number of pathologic microorganisms, and in the following 4 hours, the number of microorganisms will remain fairly constant since the rate of bacterial proliferation is about equal to the rate at which host defenses kill the bacteria. After these first 6 hours, bacteria will multiply

Table 4.3 Common microorganisms causing hip and knee prosthetic joint infection modified from Tande et al. Clinical Microbiology Reviews 2014 [5]

Infection	All time periods (%)	Early infection (%)
Staphylococcus aureus	27	38
Coagulase-negative *Staphylococcus*	27	22
Streptococcus species	8	4
Enterococcus species	3	10
Aerobic Gram-negative bacilli	9	24
Anaerobic bacteria	4	3
Culture negative	14	10
Polymicrobial	15	31
Other	3	

exponentially [32, 33]. The first 6 hours are often referred to as the "Golden Period." The administration of prophylactic antibiotics (discussed further in Chap. 5) extends this "Golden Period," and decreases bacterial growth, thus decreasing the probability of postoperative infection and success of biofilm formation and maturation [33, 34].

4.2.3   Role of Biofilm and Mechanisms of Resistance

The majority of all human infections are thought to be related to biofilm formation, and central to the pathogenesis of implant-related infection. Additionally, in the setting of PJI, when implants are retained, failure of treatment is often attributable to biofilm formation [35]. Biofilms are complex, well-structured communities of microorganisms that are encased in a self-produced extracellular matrix of polymeric substances [35]. This extracellular matrix consists of polysaccharides, proteins, and/or extracellular DNA. They can be monomicrobial or polymicrobial. Some species of bacteria will grow better together than others, and in these polymicrobial biofilms, these species may be present in varying proportions, with a different genetic makeup, even within the same species [5]. This can make them different to detect and target with antimicrobial therapy. Biofilms can be present in different forms: they can be adherent to host tissue, adherent to implant or biomaterial surfaces, present as floating aggregates, and have even been shown to persist intracellularly [5, 35].

In the setting of PJI, biofilms form when microorganisms attach to a protein-conditioned implant surface. All orthopedic implants are susceptible to biofilm attachment, which can occur either intraoperatively or at any time point postoperatively. Biofilm growth occurs in stages. The first involves attachment to the implant surface. Next, accumulation occurs, which involves interactions between bacterial cells including multilayer cellular proliferation and cell-to-cell adhesion leading to the formation of microcolonies and to the initial growth of the biofilm [20]. The next stage is maturation, where a viable three-dimensional structure is formed, eventually leading to infection. The final stage is biofilm dispersion/detachment. Notably, the life cycle of the biofilm can vary depending on the organism(s) involved, and there is no clinical research available investigating how the timing of biofilm formation differs between bacterial species [35].

Biofilms protect microorganisms from the host immune system and are up to 1000 times more resistant to growth-dependent antimicrobials than free-floating, or planktonic, microorganisms for a variety of reasons [36]. Biofilms protect invading bacteria against the host immune system through impairing the activity of phagocytes and the complement system. Specifically, the extracellular matrix is highly complex, and polar mixture of polysaccharides, nucleic acids, lipids, and proteins creates an environment that protects the bacteria from

various stresses, including the host immune system and antimicrobial exposure [37, 38]. One study investigated the potential mechanism of antibiotic resistance in *Pseudomonas aeruginosa* and found that while tobramycin and ciprofloxacin were able to penetrate the biofilm effectively, they were only effective in the oxic region of bacterial metabolic activity. Thus, oxygen limitation and decreased metabolic activity were correlated with antibiotic tolerance in this specific biofilm [39]. Other experimental studies have investigated the role of the metabolic state of biofilm and its contribution to their resistance. They found that cells in nutrient-depleted zones of the biofilm may enter into a stationary phase, where they replicate less frequently and thus are less affected by antibiotics [40, 41]. Additionally, there are bacterial subpopulations present, known as "persisters," which are resistant to antimicrobials. Experimental studies have shown that biofilm microorganisms undergo a higher rate of mutation than those in the planktonic state. This results in a tenfold increase in the efficiency of transferring plasmids with antibiotic resistance genes, when exposed to a concentration of antibiotic that is below the lethal concentration [38, 42]. Another challenge posed by biofilm formation is the difficulty in identifying the infectious organism(s). Particularly in cases of delayed or late-onset infections, the microorganism may be concentrated on the surface of the prosthesis, diminishing the sensitivity of conventional microbiologic culture methods such as joint aspiration. This can subsequently lead to failure in identifying the infecting organism and may cause challenges with antibiotic management, and efficacy in ultimately eliminating the infection [35].

Given the clinical challenges posed by biofilm formation, specifically the substantial burden on the patient and healthcare system, significant effort has been put into better understanding the many clinical aspects of biofilms, with regard to prevention and treatment. To this end, in 2018, the Biofilm Workgroup met at the International Consensus Meeting on Musculoskeletal Infection and published a consensus of the best available data on management of patients afflicted with implant-related bone and joint infections. Recent areas of research interest have been biofilm prevention and eradication in PJI. Some studies have investigated whether surfaces could be modified to inhibit biofilm formation. Properties of materials and implants that are known to affect the timing and robustness of established biofilms include surface charge, chemistry, hydrophilicity, microtopography, and porosity. However, studies investigating surface modification found to have a positive effect in vitro have not been translated to the clinical setting. To date, there is no known surface that cannot be colonized by biofilm-forming bacteria. In effect, bacteria can form biofilm on almost all prosthetic and biological surfaces [43–46]. There are also ongoing investigations on disrupting bacterial communication to inhibit biofilm formation, and the use of bacteriophages for the treatment of multidrug-resistant PJI [47, 48].

4.3 How to Diagnose PJI

4.3.1 Definition Criteria in Diagnosing PJI

Clinical signs of infection include systemic symptoms, such as fevers or chills, and local symptoms, such as pain, erythema, edema, prolonged joint effusion, and wound dehiscence. Chronic infections may be difficult to distinguish from aseptic failure, as patients may be less symptomatic in these cases. More definitive clinical signs of infection include a sinus tract or visible purulence about the prosthesis [20]. The diagnosis of PJI and how it is defined is based on a combination of clinical findings, laboratory results, culture data, histopathology evaluation, radiographic results, and intraoperative findings. There is no single test available that can definitively diagnose PJI with sufficient accuracy. Over the past years, various organizations and societies, including the American Academy of Orthopaedic Surgeons (AAOS), Musculoskeletal Infection Society (MSIS) [49], International Consensus Meeting (ICM) [50, 51], European Bone and Joint Infection Society (EBJIS) [52], PRO-IMPLANT Foundation, and Infectious Diseases Society of America (IDSA) [53], have published definition criteria and/or clinical recommendations for diagnosing PJI. In general, the steps of PJI diagnosis involve determining whether or not the joint is infected, identifying the infecting microorganism(s), and then determining an antimicrobial treatment plan [5].

The first widely adopted, standardized definition of PJI was published in 2011 by MSIS. According to these criteria, if one of the two major criteria were met (a sinus tract communicating with the prosthesis is present, or if a pathogen is isolated by culture from at least two separate tissue or fluid samples obtained from the affected prosthetic joint), or four of the six minor criteria are met, then PJI could be definitively diagnosed (Table 4.4) [49]. Of note, the authors acknowledged that these criteria may not be met in cases of low-grade infections such as those caused by *Cutibacterium* species. Additionally, these criteria did not include cutoffs for serum or synovial lab markers [49]. In 2013, these criteria were modified as part of the ICM on PJI to include acceptable thresholds for minor criteria based on the acuity of the infection. Additionally, this group added leukocyte esterase as a minor criterion (Table 4.5) [50].

These aforementioned widely used guidelines were largely generated based on previously existing data and expert opinions, but were not validated. Additionally, in recent years, numerous additional markers and molecular techniques have been evaluated and become more available for widespread use. These include serum D-dimer, synovial C-reactive protein (CRP), synovial leukocyte esterase (LE), synovial alpha-defensin, and next-generation sequencing [54–61]. For these reasons, in 2018, Parvizi et al. performed a multi-institutional study to generate an evidence-based, weight-adjusted scoring system (Table 4.6) for the definition of PJI of the hip and knee, and to validate it on an external cohort of patients [51]. This was also based on current AAOS guidelines for the diagnosis of PJI (Appendix 1) [62].

Table 4.4 2011 MSIS criteria for diagnosing PJI

2011 Musculoskeletal Infection Society (MSIS) criteria for diagnosing PJI
(1) There is a sinus tract communicating with the prosthesis; or
(2) A pathogen is isolated by culture from at least two separate tissue or fluid samples obtained from the affected prosthetic joint; or

(3) Four of the following six criteria exist:
  (a) Elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration
  (b) Elevated synovial leukocyte count
  (c) Elevated synovial neutrophil percentage (PMN%)
  (d) Presence of purulence in the affected joint
  (e) Isolation of a microorganism in one culture of periprosthetic tissue or fluid
  (f) Greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at x400 magnification

PJI may be present if fewer than four of these "minor" criteria are met

Adapted from Parvizi et al. Clin Orthop Relat Res 2011 [49]

In these updated criteria, two positive cultures of the same organism or the presence of a sinus tract are considered as major criteria, and diagnostic of PJI.

Serum D-dimer, synovial alpha-defensin, and synovial CRP were added to pre-operative minor criteria. These guidelines also identified criteria for intraoperative diagnosis in the case of inconclusive pre-op scores or a dry tap, which includes the preoperative score, positive histology, purulence, and a single positive culture (Table 4.6). Minor criteria were assigned relative weights. These criteria were used validated on an external cohort of 222 patients with PJI who subsequently failed with reinfection and 200 aseptic patients. This new definition was compared to the 2011 MSIS criteria and 2013 ICM criteria and demonstrated improved sensitivity (97.7%) and similar specificity (99.5%) (Table 4.7). While these criteria have since been validated in literature in additional cohorts of patients, it is not without limitations, as noted by the authors [63]. These new criteria were developed and validated on a cohort of patients with chronic PJI, not acute PJI. Conventional culture techniques were used, which did not include sonication or next-generation sequencing. The same criteria were applied to both knees and hips, despite some studies noting differences in thresholds for synovial markers between the PJI of the hip and knee, and these criteria may be inaccurate in patients with special conditions such as inflammatory arthropathy flares, local tissue reactions, and crystalline deposition arthropathy and those under antibiotic treatment [63].

.

The European Bone and Joint Infection Society (EBJIS) proposed diagnostic PJI criteria with positive infection in the presence of greater than or equal to one of the following criteria: purulence around the prosthesis or sinus tract, increased synovial fluid leukocyte count, positive histopathology, or confirmatory microbial growth in synovial fluid, periprosthetic tissue, or sonication culture [64]. More recently the EBJIS published a consensus document in 2019 in collaboration with a number of other European societies [52]. The 2019 guidelines are notable for an increased focus on using imaging to assist in the diagnosis of PJI. In their proposed diagnostic

Table 4.5 2013 ICM criteria for diagnosing PJI. (Adapted from Parvizi et al. Journal of Arthroplasty 2014 [50])

2013 International Consensus Meeting Definition of Periprosthetic Joint Infection	
Major Criteria	Two positive periprosthetic cultures with phenotypically identical organisms, OR A sinus tract communicating with the joint, OR
Minor Criteria	1) Elevated serum C-reactive protein (CRP) AND erythrocyte sedimentation rate (ESR) 2) Elevated synovial fluid white blood cell (WBC) count OR ++change on leukocyte esterase test strip 3) Elevated synovial fluid polymorphonuclear neutrophil percentage (PMN%) 4) Positive histological analysis of periprosthetic tissue 5) A single positive culture
PJI may be present without meeting these criteria, specifically in the case of less virulent organisms	

Threshold for minor diagnostic criteria		
Criterion	Acute PJI (<90 days)	Chronic PJI (>90 days)
Erythrocyte Sedimentation Rate (mm/hr)	Not helpful, No threshold was determined	30
C-Reactive Protein (mg/L)	100	10
Synovia White Blood Cell Count (cells/µl)	10,000	3,000
Synovial Polymorphonuclear (%)	90	80
Leukocyte Esterase	+ or ++	+ or ++
Histological Analysis of Tissue	>5 neutrophils per high power field in 5 high power fields (x400)	Same as acute

flowchart for suspected PJI, they first recommend a workup with blood cultures, standard labs, and radiographic imaging. If suspicion persists, then they recommend bone or soft tissue biopsy or aspiration under imaging guidance, followed by advanced imaging, which may consist of MRI or different nuclear medicine examinations. While these guidelines do not provide specific diagnostic criteria, they bring attention to the potential role of different advanced imaging techniques and nuclear medicine procedures in diagnosing PJI (Table 4.8). This group proposes a specific clinical path to undertake when using nuclear medicine procedures to diagnose suspected PJI, in particular, to use WBC scans with or without bone marrow scans (within 2 years of surgery), and three-phase bone scans or FDG-PET scans

Table 4.6 2018 ICM criteria for diagnosing PJI and lab value thresholds. (Adapted from Parvizi et al. Journal of Arthroplasty 2018 [51])

New International Consensus Meeting Definition of Periprosthetic Joint Infection	
Major criteria **At least one of the following**	- Two positive cultures of the same organism OR - Sinus tract with evidence of communication to the joint or visualization of the prosthesis
Pre-operative Minor Criteria ≥ 6 Infected 2-5 Possibly infected 0-1 Not infected	- Elevated serum CRP or D-Dimer (2) - Elevated serum ESR (1) - Elevated synovial WBC count or synovial leukocyte esterase (3) - Positive synovial alpha defensin (3) - Elevated synovial PMN % (2) - Elevated synovial CRP (1)
Inconclusive pre-op score or dry tap ≥ 6 Infected 4-5 Inconclusive ≤ 3 Not infected	- Preoperative score - Positive histology (3) - Positive purulence (3) - Single positive culture (2)

Proposed Lab Value Thresholds		
Marker	**Chronic (>90 days)**	**Acute (<90 days)**
Serum CRP (mg/dL	1.0	10
Serum D-Dimer (ng/mL)	860	860
Serum ESR (mm/h)	30	-
Synovial WBC count (cells/microliter)	3000	10,000
Synovial PMN (%)	80	90
Synovial CRP (mg/L)	6.9	6.9
Synovial alpha-defensin (signal to cutoff ratio)	1.0	1.0

(>2 years from surgery) to assist in diagnosing PJI (Fig. 4.1) [52]. The authors do recognize the limitations of these tests, particularly with regard to their availability and cost.

Table 4.7 Performance of new ICM definition (2018) compared to 2011 MSIS and 2013 ICM definitions of PJI

Performance of the new International Consensus Meeting (ICM) definition compared to MSIS and 2013 ICM criteria		
Criteria	Sensitivity	Specificity
MSIS (2011)	79.3%	99.5%
ICM (2013)	86.9%	99.5%
New definition (2018)	97.7%	99.5%

Adapted from Parvizi et al. Journal of Arthroplasty 2018 [51]

4.4 Tests and Tools for Diagnosing PJI

4.4.1 Serum-Based Markers

4.4.1.1 ESR and CRP

Currently, inflammatory markers including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are routinely collected during the initial PJI workup [65]. While white blood cell count is also often collected as part of routine complete blood count (CBC) collection, studies have shown that elevated WBC has poor sensitivity (45%) and specificity (87%) for diagnosing PJI and is not currently recommended for PJI diagnosis [51, 66]. CRP and ESR are currently recommended as first-line tests for PJI workup and are included in multiple widely used diagnostic criteria and consensus guidelines including the most recent ICM guidelines and AAOS Clinical Practice Guidelines. Both tests are widely available and inexpensive. However, neither of these tests is very sensitive or specific, with CRP having slightly higher sensitivity and specificity compared to ESR. Sensitivities for ESR and CRP have been reported to be 75% and 88%, respectively. Specificities for ESR and CRP have been reported to be 70% and 74%, respectively [66]. However, used in combination, these lab tests can be useful in ruling out PJI if both values are within normal limits (30 mm/h for ESR and 10 mg/liter for CRP) [67]. Unfortunately, studies have shown that the specificity of these tests in combination, where one or both values is positive, is low [5, 67]. Additionally, these values can vary significantly depending on the presence of concomitant systemic inflammatory diseases, and depending on the time of infection since surgery. A study by Alijanipour et al. investigated threshold values for infection for ESR and CRP in early postoperative compared to late-chronic PJI. They found that optimal thresholds were higher than conventional thresholds for both early and late PJIs and that in late infections, optimal thresholds for ESR and CRP differed between hips and knees [68]. Given the limitations of these commonly used tests, recent studies have looked into developing alternative serum biomarkers that may offer improved sensitivity and specificity.

Table 4.8 Use of advanced imaging and nuclear medicine techniques in the diagnosis of PJI

Pros and cons of advanced imaging techniques		
	Pros	Cons
Computed tomography (CT)	– Widely available with medium cost – Can be used for guided aspiration and bone biopsy	– Striking artifacts due to prosthesis – Lower diagnostic accuracy than MRI – Radiation exposure
Magnetic resonance imaging (MRI)	– High diagnostic accuracy with new sequences without prosthesis interference – Widely available with medium cost – Radiation-free	Peri-implant edema may result in false-positive findings
Advanced nuclear medicine techniques		
	Pros	Cons
99mTc-MDP/HDP bone scan	– High sensitivity – Useful as screening method in chronic infections – Widely available and low cost	– Low specificity – Moderate radiation exposure
99mTc-anti-granulocyte scan (IgG/Fab AGA)	– High sensitivity and specificity – Widely available and medium cost – Often coupled with bone marrow scan and/or bone scan	– Possible contraindications for IgG and HAMA induction – Moderate radiation exposure
99mTc-HMPAO/111In-oxine-WBC scan	– High sensitivity and specificity – Poor availability and medium cost – Often coupled with bone marrow scan SPECT/CT images improve accuracy	– Moderate radiation exposure – Always requires a late acquisition – Blood manipulation – Needs an approved laboratory and method and trained personnel
[18F] FDG-PET/CT	– High sensitivity	– Low specificity – High radiation exposure – Difficult interpretation of images – Poor availability and high cost

Adapted from Signor et al. Eur J Nucl Med Mol Imaging 2019

4.4.1.2 D-dimer and Fibrinogen

One serum biomarker that has shown promise is D-dimer. The increased fibrinolytic activity and generation of by-products such as D-dimer in systemic and local infections are thought to localize the infecting organisms or inflammatory cells and thus prevent them from causing systemic damage. During this process D-dimer "leaks" into the circulation and can thus be measured. Historically, D-dimer was used as a screening test for detecting venous thromboembolism (VTE), but was largely abandoned due to its poor accuracy. In more recent years, D-dimer has gained attention for its role in predicting poor outcomes in sepsis and bacteremia [69–71]. The first
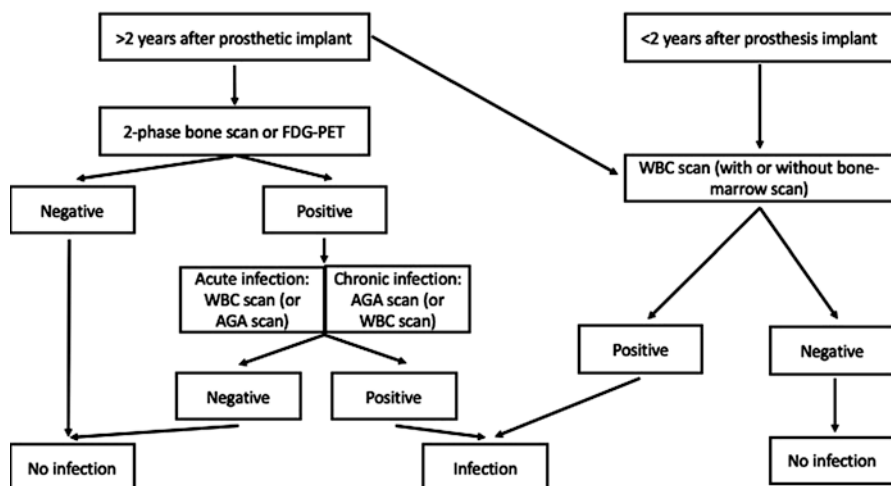
Fig. 4.1 Proposed path when nuclear medicine procedures are used to aid in diagnosis of PJI. (Adapted from Signore et al. Eur J Nucl Med Mol Imaging 2019)

study to show its promise as a marker for diagnosing PJI was reported by Shahi et al. in 2017 who found that serum D-dimer outperformed both ESR and serum CRP in detecting PJI. D-dimer specificity was 93% and sensitivity was 89%. In comparison ESR and CRP had a specificity of 78% and 80% and a sensitivity of 73% and 79%, respectively. A prospective study of 122 patients (67 patients with aseptic failure and 55 with chronic PJI) who underwent revision TKA or THA and diagnosed with PJI per MSIS criteria was analyzed to investigate the sensitivity and specificity of D-dimer in combination with CRP or ESR to detect PJI [72]. They reported that the optimal threshold value for D-dimer in diagnosing chronic PJI was 1170 ng/mL with a sensitivity of 92% and specificity of 75%, greater than the reported sensitivities and specificities for ESR and CRP in this study. Additionally, the combination of D-dimer and CRP tests demonstrated a sensitivity of 98% and negative predictive value of 96% for diagnosing chronic PJI, though had a low specificity of 42%. Elevation of all three biomarkers yielded similar results [72]. Studies such as this suggest a role for D-dimer in confidently ruling out PJI when used in combination with other serum biomarkers.

Fibrinogen is another coagulation-related indicator in plasma that has recently gained attention as a potential biomarker for PJI diagnosis. Fibrinogen is a glycoprotein which functions to stop excessive bleeding through the formation of a fibrin-based blood clot after its conversion by thrombin to fibrin. It is also a type of acute-phase protein [73]. A recent study by Li and colleagues retrospectively reviewed a total of 439 revision total hip and knee arthroplasty cases (76 PJI and 363 non-PJI) and found that the optimal threshold for plasma fibrinogen in diagnosing PJI was 4.01 g/L, which showed a sensitivity of 76% and a specificity of 86%, which were similar to the diagnostic values of ESR and CRP in this study.

However, they also examined plasma D-dimer and reported a fairly low sensitivity and specificity of only 64% and 65%, respectively, at a threshold level of 1.25 micrograms/mL. They theorize that this discrepancy in reported D-dimer reliability may be related to differences in the demographics of the patient populations [74]. Another recent meta-analysis of 7 studies with 1374 patients total compared D-dimer versus fibrinogen in the diagnosis of PJI and reported a pooled sensitivity of 84% and pooled specificity of 80% for fibrinogen. This group concluded that plasma fibrinogen is comparable to CRP and ESR in diagnosing PJI, and all are better than serum D-dimer. Once again, literature has shown that D-dimer levels have been shown to be different in different races, and it is unknown if this is also the case for fibrinogen [75]. Regardless, fibrinogen has shown promise in these early studies, and warrants further investigation for its potential for a routine role in the diagnosis of PJI.

4.4.1.3 Procalcitonin

Another serum biomarker that has recently been investigated for its diagnostic potential is procalcitonin (PCT). PCT is produced by neuroendocrine cells and thyroid parafollicular cells, and is typically extremely low in patients without infection. A study by Bottner et al. found that a procalcitonin level > 0.3 ng/ml, determined by receiver operating characteristic curve analysis, was 98% specific, but only 33% sensitive [76]. Another recent study performed a meta-analysis of 18 studies encompassing 1835 patients and found that the pooled sensitivity was 58% for procalcitonin and specificity of 95% for infection [77]. However more, studies need to be conducted on the utility PCT specifically for diagnosing PJI compared to other infections.

4.4.1.4 Interleukin-6 (Serum and Synovial)

Interleukin-6 (IL-6) is another serum biomarker of interest and is produced by stimulated monocytes and macrophages. Serum IL-6 can increase in the setting of infection, surgery, and trauma. In the setting of aseptic prosthetic loosening, serum IL-6 has been shown to decrease to normal levels within 48 hours after surgery. Serum IL-6 stimulates the release of CRP. Given the early rise of IL-6 and quick return to normal levels IL-6, this serum biomarker may be more sensitive in detecting an inflammatory response than other serum biomarkers [78, 79]. A recent meta-analysis that included 17 studies (Table 4.9) found that the pooled sensitivity and specificity for serum IL-6 were 72% and 89%, respectively. Synovial IL-6 has been shown to have a slightly higher diagnostic value for PJI, with a pooled sensitivity of 76%% and specificity of 91% (Table 4.10) [80]. IL-6, either from synovium or serum, shows promise based on existing studies, but is not yet widely used given its reported variability and lack of consistency in literature [81, 82].

4.4.2 Synovial Fluid-Based Markers

4.4.2.1 White Blood Cell Count and Neutrophil Differential

Assessment of synovial fluid white blood cell (WBC) count and neutrophil differential (PMN%) both has an important role in the diagnosis of PJI. Both of these parameters are included in the minor criteria of the ICM criteria, and many studies have demonstrated their usefulness in diagnosing PJI [83–86]. Depending on the timing of infection, there are variable thresholds that have been identified. For the acute period, which is generally considered less than 6 weeks postoperatively, a threshold of greater than 10,000 cells per microliter for synovial WBC count and a threshold >90% for synovial PMN% have been recommended to diagnose PJI. For chronic PJI, considered greater than 6 weeks from surgery, a threshold of >3000 cells per microliter for synovial WBC count and threshold of >80% for PMN% are recommended for diagnosis [87, 88]. These are the cutoffs that are included in the most recent ICM criteria for PJI diagnosis. Using these thresholds, sensitivities of 86% and 86% for WBC count and PMN%, respectively, and specificities of 83% and 81% for WBC count and PMN%, respectively, have been reported [60].

Some studies have suggested that there should be different thresholds for knee arthroplasties versus hip arthroplasties, with hips possibly having higher thresholds. A study of 201 THA with 55 PJIs found that a synovial WBC count of 4200 cells per microliter provided a sensitivity and specificity of 84% and 93%, respectively, and a PMN% of 80% had a sensitivity and specificity of 84% and 82%, respectively [89]. Another study reported an optimal threshold 1715 cells per microliter for PJI in THA, but only had 27 patients [83]. For PJI in TKA, one of the largest studies to date analyzed 429 knees with 161 PJIs and found that the optimal threshold for infection was 1100 cells per microliter and > 64% for the PMN%. This resulted in a negative predictive value of 98.2% when both were below cutoff values, and confirmed infection in 98.6% of cases when both were greater than their cutoff values [86]. Given some of these conflicting results, further investigation is warranted to

Table 4.9 Serum IL-6 sensitivities and specificities in diagnosing prosthetic joint infection

Author	Year	# Patients	Sensitivity	Specificity
Di Cesare et al. [120]	2005	58	100%	95%
Bottner et al. [76]	2007	78	95%	88%
Buttaro et al. [121]	2010	69	36%	94%
Worthington et al. [122]	2010	46	81%	77%
Abou El-Khier et al. [123]	2013	40	100%	90%
Glehr et al. [124]	2013	84	81%	67%
Gollwitzer et al. [125]	2013	35	47%	95%
Randau et al. [82]	2014	120	79%	58%
Ettinger et al. [126]	2015	98	80%	88%
Gallo et al. [127]	2018	240	87%	89%
Total		868	79%	84%

Table 4.10 Synovial IL-6 sensitivities and specificities in diagnosing prosthetic joint infection

Author	Year	# Patients	Sensitivity	Specificity
Deirmengian et al. [128]	2010	51	50%	100%
Deirmengian et al. [98]	2014	95	90%	97%
Frangiamore et al. [129]	2016	90	81%	97%
Gollwitzer et al. [125]	2013	35	60%	95%
Jacovides et al. [130]	2011	74	87%	100%
Lenski et al. [131]	2014	40	90%	95%
Nilsdotter-Augustinsson et al. [132]	2007	131	68%	93%
Randau et al. [82]	2014	120	63%	86%
Gallo et al. [127]	2018	240	68%	95%
Total		876	73%	95%

determine if different cutoffs for PJI in knees and hips are warranted. Additionally, there are certain clinical scenarios that will alter synovial WBC count and PMN differential that clinicians should be aware of. The use of antibiotics, traumatic aspirations, and failed metal on metal bearing or corrosion reactions can all influence synovial WBC count and PMN%, and these results should be interpreted with caution [90–92]. In cases of failed metal on metal bearing or corrosion reactions, a manual synovial fluid WBC is recommended to obtain more accurate results. In patients with inflammatory arthritis, optimal cutoffs for PJI were found to be similar to those without inflammatory arthritis [93].

4.4.2.2   Synovial CRP

Synovial CRP enhances complete activation and phagocytosis, and may also have a role in diagnosis of PJI (Table 4.11). It is currently included as a minor criterion in the most recent ICM criteria for PJI diagnosis. Pooled data show a sensitivity of 86% and specificity of 90% [80]. Some studies have suggested that synovial CRP is superior to serum CRP [94], but a study by Tetreault reported that serum and synovial CRP were not significantly different with regard to sensitivity and specificity [95]. A recent study by Stone et al. found that synovial alpha-defensin in combination with synovial CRP, where a positive result was defined as a positive CRP or a positive alpha-defensin, demonstrated very high sensitivity of 91% and a specificity of 79% [96].

4.4.2.3   Alpha-Defensin

Alpha-defensin is a synovial biomarker that has shown promise in diagnosing PJI in recent studies (Table 4.12). Alpha-defensin is an antimicrobial peptide secreted by human neutrophils in response to the presence of a pathogen. It acts via permeabilization of microbial membranes and is unaffected by prior administration of

antibiotics and has been shown to rise even in response to low-virulence organisms [58, 97, 98]. Alpha-defensin is detected either with an alpha-defensin test kit or laboratory-based alpha-defensin enzyme-linked immunosorbent assay (ELISA). A meta-analysis by Ahmad et al. in 2018 analyzed the reliability for various synovial biomarkers including alpha-defensin ELISA testing and reported a pooled sensitivity and specificity of 97% and 97%, respectively, with some earlier studies reporting up to 100% sensitivity and specificity with alpha-defensin ELISA testing. More recent studies, however, suggest that alpha-defensin ELISA may not be as sensitive as initial studies suggested, with reports ranging between 78.2% and 97% in recently published literature [96, 99–101]. In a study by Kleiss et al., 22% of alpha-defensin ELISA tests were false-negative, mostly in cases of coagulase-negative staphylococcus [99]. Further work is warranted on how different types of bacteria may affect the accuracy of alpha-defensin testing. As previously mentioned, another method of evaluating alpha-defensin levels is the alpha-defensin lateral flow test. This type of testing enables the detection of alpha-defensin in synovial fluid "in situ," and the response is available in 10 minutes, which is much quicker than the ELISA test. Sensitivities reported in literature range from 65% to 98%, with a pooled value of 80%, and specificities range from 93% to 100%, with a pooled value of 89% (Table 4.13) [80, 102]. Comparative studies have demonstrated that these alpha-defensin lateral flow tests are not as reliable as ELISA tests. Nonetheless, the lateral flow tests are specific, and allow for quick result turnaround time. One of the main challenges with alpha-defensin tests is the associated expense. Further cost-effectiveness studies will examine whether these associated costs are justifiable.

Table 4.11 Synovial CRP sensitivities and specificities in diagnosing prosthetic joint infection

Author	Year	# Patients	Sensitivity	Specificity
Buttaro et al. [133]	2015	76	89%	94%
Deirmengian et al. [58]	2014	95	95%	89%
Jacovides et al. [130]	2011	74	86%	97%
Omar et al. [61]	2015	89	93%	92%
Parvizi et al. [94]	2012	66	70%	100%
Parvizi et al. [94]	2012	66	84%	97%
Parvizi et al. [134]	2012	63	83%	94%
Ronde-Oustau et al. [135]	2014	30	100%	82%
Ronde-Oustau et al. [135]	2014	30	90%	91%
Tetreault et al. [95]	2014	150	88%	85%
Vanderstappen et al. [136]	2013	44	96%	82%
Vanderstappen et al. [136]	2013	44	88%	89%
De Vecchi et al. [137]	2016	129	81%	94%
De Vecchi et al. [138]	2018	66	87%	97%
Kim et al. [139]	2017	197	100%	90%
Sousa et al. [140]	2017	55	78%	94%
Plate et al. [141]	2019	192	88%	82%
Total		1466	88%	91%

Table 4.12 Alpha-defensin ELISA sensitivities and specificities in diagnosing prosthetic joint infection

Author	Year	# Patients	Sensitivity	Specificity
Deirmengian et al. [98]	2014	149	97%	96%
Deirmengian et al. [58]	2014	95	100%	100%
Frangiamore et al. [129]	2016	90	100%	98%
Bingham et al. [142]	2014	55	100%	95%
Deirmengian et al. [143]	2015	46	100%	100%
Shahi et al. [92]	2016	106	100%	
Bonanzinga et al. [100]	2017	156	97%	97%
Sigmund et al. [101]	2018	73	85%	98%
Stone et al. [96]	2018	183	81%	96%
Kleiss et al. [99]	2019	202	78%	96%
Total		1155	94%	97%

4.4.2.4 Leukocyte Esterase

Another synovial biomarker that has gained widespread usage is leukocyte esterase. This is an enzyme produced by activated neutrophils at the site of infection. It has traditionally been used to help diagnose urinary tract infections and is convenient, cheap (about 20 cents), and rapid (1–2-minute turnaround time). It is also included as a minor criterion in the ICM criteria for PJI. It is easily measured with a colorimetric strip (urinalysis dipstick). A meta-analysis by Wyatt et al. reported a pooled sensitivity and specificity of 81% and 97%, respectively. Reported sensitivities range from 69% to 100%, with some authors reporting increased sensitivity compared to frozen section histology and advocating for its use over frozen section histology [103]. Its ability to provide almost immediate test results is surely appealing, and its role in diagnosing PJI may continue to increase over time. One disadvantage to this technique is the possibility that blood within synovial fluid may interfere with the color change on the urinalysis test strip [104]. For this reason, it is important for surgeons to remove all blood contamination from the sample, ideally with the use of a centrifuge [88].

The aforementioned synovial fluid markers have all shown promise in assisting with diagnosis of PJI in recent literature. Lee et al. conducted a systematic review and meta-analysis with the goal of evaluating the diagnostic accuracy of synovial fluid biomarkers and to determine which has the highest diagnostic odds ratio for the diagnosis of PJI. The study compared leukocyte count, PMN%, CRP, alpha-defensin, leukocyte esterase, IL-6, IL-8, and culture and found that these all demonstrated sensitivity >80% with the exception of culture, and specificity >90%, but alpha-defensin had the high log diagnostic odds ratio compared to all other tests [54]. As was previously described, alpha-defensin, while a powerful diagnostic tool, may not be adequate to diagnose PJI alone. We support the use of a combination of these markers for the diagnosis of PJI to increase overall sensitivity and specificity.

Table 4.13 Alpha-defensin lateral flow test specificities and sensitivities in diagnosing prosthetic joint infection

Author	Year	# Patients	Sensitivity	Specificity
Kasparek et al. [144]	2016	40	67%	93%
Sigmund et al. [145]	2017	50	69%	94%
Suda et al. [146]	2017	28	77%	82%
Berger et al. [147]	2017	121	97%	96%
Balato et al. [148]	2017	51	87%	97%
Gehrke et al. [149]	2018	223	92%	100%
Sigmund et al. [150]	2019	101	69%	94%
Riccio et al. [151]	2018	73	85%	97%
De Saint Vincent et al. [152]	2018	42	88%	87%
Renz et al. [64]	2018	212	84%	96%
Tahta et al. [153]	2018	38	94%	100%
Plate et al. [154]	2018	109	90%	92%
Total		1088	83%	94%

4.4.3   Culture Diagnosis

Obtaining culture data is an essential part of the infectious workup in PJI. The AAOS Clinical Practice Guidelines states that moderate strength evidence supports the collection of synovial fluid aerobic and anaerobic cultures to aid in the diagnosis of PJI. Additionally, two positive cultures of the same bacteria are one of the two major criteria to diagnose PJI according to the most recent MSIS criteria. The information gained from these tests can inform perioperative antibiotic management, and may also impact surgical treatment, particularly in a resistant microorganism, such as methicillin-resistant *Staphylococcus aureus* (MRSA).

Typically, preoperative aspirated synovial fluid can be either directly inoculated into blood culture bottles (BCBs) at the time of collection or transported to the microbiology laboratory and placed onto liquid or solid media [5]. A meta-analysis in 2013 reported that preoperative aspiration culture for PJI has a pooled sensitivity and specificity of 72% and 95%, respectively [105]. Prior studies have investigated the improved ability of BCBs in detecting pathogens and having decreased contaminants compared to conventional placement of synovial fluid onto agar media [106–108]. One study compared the frequency of positive cultures with synovial fluid inoculated in blood culture flasks with those of intraoperative swabs or periprosthetic tissues in traditional cultures. They found that synovial fluid samples in BCBs were more sensitive (91%) and specific (100%) than standard periprosthetic tissue and swab samples cultured in standard media. BCBs are self-sustaining media, which may be more ideal for bacterial growth, and have been shown to detect the presence of infectious organisms with smaller quantities of fluid [108]. Additionally, BCBs for synovial fluid have been reported to have improved detection of infection in acute infection compared to chronic infection, likely secondary to more

planktonic bacteria present in acute infection [107]. The primary disadvantage of using BCBs compared to traditional cultures is the associated cost.

Intraoperative periprosthetic tissue cultures are another useful diagnostic tool in the workup of PJI. It is well known that it is important to obtain multiple tissue cultures intraoperatively, but the appropriate number of cultures has been an area of debate [109, 110]. A highly cited study by Atkins et al. published in 1998 recommended five to six specimens be obtained, with a cutoff of two or more yielding the same microorganism to diagnose infection. Most studies, however, used different types of culture media to periprosthetic tissue samples, which would have an impact on culture yield and accuracy. A more recent study by Peel et al. compared conventional culture techniques (aerobic and anaerobic agars and thioglycolate broth) to inoculation in BCBs and also performed statistical analyses with conventional, frequentist receiver-operating characteristic curve analysis to determine the optimal number of intraoperative tissue samples to obtain. They found that the greatest accuracy was obtained when three periprosthetic tissue specimens were obtained and collected into BCBs (92%) or four periprosthetic tissue specimens were obtained and cultured using standard plate and broth cultures (91%) [109]. This study also reports improved sensitivity with using BCBs (92%) compared to conventional agar and broth cultures (63%). The specificity between the two was similar [109]. In addition to periprosthetic cultures to aid in the diagnosis of PJI, histologic analysis intraoperatively is also a useful diagnostic tool. Histologic evaluation demonstrating acute inflammation, which is defined as neutrophilic infiltrate on fixed or frozen tissue, is suggestive of PJI. A meta-analysis involving over 3000 patients found that the presence of acute inflammation had a positive likelihood ratio of 12, and a negative likelihood ratio of 0.12, which suggests that histology may not be as useful in cases with an intermediate pretest probability for PJI [5, 111]. Another recent study investigated the MSIS microbiological and histologic criteria for PJI in 60 septic knees and 78 aseptic knees that underwent revision surgery, and found that the sensitivity and specificity of MSIS histologic criteria for PJI were 96.7% and 100% [112]. While histology may be useful in diagnosis of PJI, it is predicated on the availability of a pathologist, and is more likely limited to larger academic centers. Preoperative biopsy has also been discussed and investigated, but given the lack of superiority of preoperative biopsy cultures compared to synovial aspirate, and the additional cost, invasiveness, and associated complications, it is not usually recommended [5, 113].

4.4.4 Molecular Diagnosis Techniques: PCR and Gene Sequencing

Studies have recently investigated the role of molecular diagnostic techniques in diagnosing PJI. Different assays exist, including broad-range PCR assays, multiplex PCR, and sequencing assays. Broad-range PCR assays can detect nucleic acid

sequences conserved across many species, while multiplex PCR can detect targeted microorganisms and may include up to several dozen species. PCR, compared to intraoperative tissue culture, has potential advantages including faster result turnaround time of 4–5 hours, and ability to identify antibiotic resistance markers [114]. Most studies that have been performed have focused on broad-range PCR assays, which have shown concern for poor sensitivity [114]. Bemer et al. in 2014 published a prospective, multicenter, cross-sectional study on 264 suspected PJI cases and 35 controls and reported a sensitivity of 73% and specificity of 95%, and recommended the use of multiplex PCR or pathogen-specific PCR assays over broad-range PCR assays [114]. A recent meta-analysis of PCR using sonication prosthetic fluid included 9 studies with 1340 patients and also reported a relatively low sensitivity of 75% and specificity of 96% [115]. The majority of studies in this meta-analysis included broad-range PCR. However, even recent studies that were performed using multiplex PCR have demonstrated relatively low sensitivity, but excellent specificity (Table 4.14). Authors have hypothesized that administration of pre-sampling antibiotics, with too many genomic targets, having software with high thresholds for detecting bacterial DNA may be reasons for the poor sensitivities reported.

Despite these challenges with molecular diagnostic methods, they may have a role in culture-negative PJI (CN PJI). CN PJI has been reported to range from 27% to 55% [59, 116]. Without knowledge of the infecting organism, it becomes more difficult to treat patients, and monitor the efficacy of treatment. This is where molecular diagnostics may have a role, particularly in cases where rare bacteria that are difficult to culture are the cause of disease. A small number of studies have investigated the role of next-generation sequencing (NGS) in identifying CN PJI. NGS is a non-Sanger-based high-throughput DNA sequencing method. Unlike PCR it does not rely on a panel of PCR primer targets, but rather amplifies and characterizes all microbial DNA present within a sample. A recent study by Tarabichi et al. demonstrated that NGS was able to detect 82% (9 out of 11) of CN PJI [59]. Another study reported a 44% detection rate of potential pathogens in culture-negative PJI [117]. The primary challenges that exist with NGS are host DNA contamination and the cost of using this method. Given the associated cost of molecular methods of diagnosis, and literature that has not shown a significant difference in reliability between conventional culture methods and PCR or gene sequencing, we do not currently recommend routine use of these methods, particularly as the sole means to diagnosing PJI. As discussed, PCR and gene sequencing may be useful in selected cases of PJI where diagnosis and identification of infecting organisms remains undetermined by conventional culture methods [118], but further research and advances in this technology are needed.

A recent systematic review was conducted by Carli et al. that aimed to compare the diagnostic accuracy of serum, synovial, and tissue-based tests for chronic PJI. Overall, 83 unique PJI tests were identified, and 17 underwent meta-analysis. These included serum CRP, ESR, IL-6, PCT, WBC count, synovial alpha-defensin, leukocyte esterase strips, PMN% joint aspiration culture, tissue culture, PCR, Gram stain, swab culture, and histologic analysis. This study reports that the literature on

Table 4.14 Point-of-care multiplex PCR sensitivities and specificities in diagnosing prosthetic joint infection

Author	Year	# Patients	Sensitivity	Specificity
Hischebeth et al. [155]	2016	31	67%	100%
Prieto-Borja et al. [156]	2017	68	61%	98%
Lausmann et al. [157]	2017	60	79%	100%
Portillo et al. [158]	2012	86	96%	100%
Suren et al. [159]	2020	26	67%	91%
Morgenstern et al. [160]	2018	142	60%	89%
Total		413	72%	89%

chronic PJI tests is highly heterogeneous and at risk for bias. They did report, however, that laboratory-based alpha-defensin studies and leukocyte esterase strips (2+) outperformed all other tests with regard to test sensitivities and specificities. Other high-performing diagnostic tests included synovial CRP, WBC count, and PMN% [119].

4.5   Conclusions

The incidence of PJI after primary hip or knee arthroplasty ranges from 0.5% to 2.5% [5–8] and accounts for up to 25% of the revision surgeries performed [9, 10]. There are multiple classification schemes that have been proposed, though the clinical classification used most frequently is based on the timing of infection since surgery and includes acute, chronic/delayed, and late/acute hematogenous infection [14–18]. It is also important to consider the condition of the patient (host) when classifying PJI which will help guide patient treatment and provide insight into patient prognosis. The majority of PJI occur through inoculation of microorganisms intraoperatively [5, 17]. Another mechanism of colonization is through direct spread of infection, and a third mechanism is hematogenous seeding of the prosthesis from a distant primary focus. When microorganisms first contact with the prosthesis intraoperatively, they immediately adhere to the implant surface and begin forming a biofilm. Studies have demonstrated that prevalence of infection during these initial hours depends on the number of bacteria present and the immune status of the host. The first 6 hours postoperatively are often referred to as the "Golden Period." The administration of prophylactic antibiotics extends this "Golden Period," and decreases the probability of postoperative infection and success of biofilm formation [33, 34]. Biofilms are complex, well-structured communities of microorganisms that are encased in a self-produced extracellular matrix of polymeric substances [35]. In the setting of PJI, biofilms form when microorganisms attach to a protein-conditioned implant surface. Biofilms protect invading bacteria against the host immune system through impairing the activity of phagocytes and the complement system. Biofilm may also enter into a stationary phase, where they replicate less

frequently and thus are less affected by antibiotics [40, 41]. Another challenge posed by biofilm formation is the difficulty in identifying the infectious organism(s). Particularly in cases of delayed or late-onset infections, the micro-(s) may be concentrated on the prosthesis, diminishing the sensitivity of culture methods such as joint aspiration [20].

The diagnosis of PJI and how it is defined is based on a combination of clinical findings, laboratory results, culture data, histopathology evaluation, radiographic results, and intraoperative findings. There is no single test available that can definitively diagnose PJI with sufficient accuracy. Over the past years, various organizations and societies have created definition criteria to help determine whether or not a joint is infected. The ICM criteria and MSIS criteria are the most commonly cited, and take multiple clinical and laboratory factors into consideration. A large number of serum, synovial, tissue, and molecular-based diagnostic tests are available to clinicians to aid in diagnosing PJI, all with varying levels of reliability. ESR and CRP are less sensitive and specific tests that are often obtained as first-line serum-based tests given their availability and quick turnaround time. Other serum biomarkers have shown promise in recent years including D-dimer, fibrinogen, IL-6, and procalcitonin, particularly when used in combination to diagnose PJI. Synovial fluid biomarkers are also frequently obtained – WBC count and PMN% play an important role in the workup of PJI, with significant research performed on these biomarkers. Other newer synovial biomarkers such as alpha-defensin, IL-6, and leukocyte esterase have also shown high sensitivity and specificity in diagnosing PJI. Synovial alpha-defensin and leukocyte esterase, in particular, have shown very high sensitivities and specificities across many studies. Culture diagnosis is an important part of the PJI workup – preoperative aspirates should be collected in blood culture bottles and intraoperative tissue cultures collected for eventual determination of infecting microorganism to help guide antibiotic treatment. More recently, research has been done on molecular diagnostic techniques including PCR and gene sequencing to diagnose PJI, with mixed results. However, one area that research suggests is diagnosing culture-begative PJI.

Appendix 1: AAOS Clinical Practice Guidelines for Diagnosing Prosthetic Joint Infection (Adapted from AAOS Clinical Practice Guidelines)

1. In the absence of reliable evidence about risk stratification of patients with a potential periprosthetic joint infection, testing strategies should be planned according to whether there is a higher or lower probability that a patient has a hip or knee periprosthetic infection.
2. We recommend erythrocyte sedimentation rate AND C-reactive protein testing for patients assessed for periprosthetic joint infection.

3. We recommend joint aspiration of patients being assessed for periprosthetic knee infections who have abnormal erythrocyte sedimentation rate AND/OR C-reactive protein results. We recommend that the aspirated fluid be sent for microbiologic culture and synovial fluid white blood cell count and differential.

4. We recommend a selective approach to aspiration of the hip based on the patient's probability of periprosthetic joint infection and the results of the erythrocyte sedimentation rate (ESR) AND C-reactive protein (CRP). We recommend that the aspirated fluid be sent for microbiologic culture and synovial fluid white blood cell count and differential.

5. We suggest a repeat hip aspiration when there is a discrepancy between the probability of periprosthetic joint infection and the initial aspiration culture result.

6. In the absence of reliable evidence, patients judged to be at lower probability for periprosthetic hip infection and without planned reoperation who have abnormal erythrocyte sedimentation rates OR abnormal C-reactive protein levels should be re-evaluated within 3 months. We are unable to recommend specific diagnostic tests at the time of this follow-up.

7. In the absence of reliable evidence, a repeat knee aspiration should be performed when there is a discrepancy between the probability of periprosthetic joint infection and the initial aspiration culture result.

8. We suggest patients be off of antibiotics for a minimum of 2 weeks prior to obtaining intra-articular culture.

9. Nuclear imaging (labeled leukocyte imaging combined with bone or bone marrow imaging, FDG-PET imaging, gallium imaging, or labeled leukocyte imaging) is an option in patients in whom diagnosis of periprosthetic joint infection has not been established and who are not scheduled for reoperation.

10. We are unable to recommend for or against computed tomography (CT) or magnetic resonance imaging (MRI) as a diagnostic test for periprosthetic joint infection.

11. We recommend against the use of intraoperative Gram stain to rule out periprosthetic joint infection.

12. We recommend the use of frozen sections of peri-implant tissues in patients who are undergoing reoperation for whom the diagnosis of periprosthetic joint infection has not been established or excluded.

13. We recommend that multiple cultures be obtained at the time of reoperation in patients being assessed for periprosthetic joint infection.

14. We recommend against initiating antibiotic treatment in patients with suspected periprosthetic joint infection until after cultures from the joint have been obtained.

15. We suggest that prophylactic preoperative antibiotics not be withheld in patients at lower probability for periprosthetic joint infection and those with an established diagnosis of periprosthetic joint infection who are undergoing reoperation.

References

1. Kurtz SM, Lau E, Ong K, Zhao K, Kelly M, Bozic KJ. .(2009). Future young patient demand for primary and revision joint replacement: national projections from 2010 to 2030. Clinical Orthopaedics and Related Research ;467(10):2606–12. 2009 Oct; Epub 2009/04/11.

2. Kurtz S, Mowat F, Ong K, Chan N, Lau E, Halpern M. Prevalence of primary and revision total hip and knee arthroplasty in the United States from 1990 through 2002. The Journal of Bone and Joint Surgery American Volume 2005;87(7):1487–97. 2005 Jul; Epub 2005/07/05.

3. Maradit Kremers H, Larson DR, Crowson CS, Kremers WK, Washington RE, Steiner CA, et al. Prevalence of Total Hip and Knee Replacement in the United States. The Journal of Bone and Joint Surgery American Volume. 2015;97(17):1386–97. 2015 Sept 2; Epub 2015/09/04.

4. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. The Journal of Bone and Joint Surgery American Volume. 2007;89(4):780–5. 2007 Apr; Epub 2007/04/04.

5. Tande AJ, Patel R. Prosthetic joint infection. Clinical Microbiology Reviews 2014;27(2):302–45. Epub 2014/04/04.

6. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. The Journal of Arthroplasty. 2012;27(8 Suppl):61–5.e1. 2012 Sep; Epub 2012/05/05.

7. Dale H, Fenstad AM, Hallan G, Havelin LI, Furnes O, Overgaard S, et al. Increasing risk of prosthetic joint infection after total hip arthroplasty. Acta Orthopaedica 2012;83(5):449–58. 2012 Oct; Epub 2012/10/23.

8. Kurtz SM, Ong KL, Lau E, Bozic KJ, Berry D, Parvizi J. Prosthetic joint infection risk after TKA in the Medicare population. Clinical Orthopaedics and Related Research 2010;468(1):52–6. 2010 Jan; Epub 2009/08/12.

9. Parvizi J, Pawasarat IM, Azzam KA, Joshi A, Hansen EN, Bozic KJ. Periprosthetic joint infection: the economic impact of methicillin-resistant infections. The Journal of Arthroplasty 2010;25(6 Suppl):103–7. 2010 Sep; Epub 2010/06/24.

10. Bozic KJ, Kurtz SM, Lau E, Ong K, Chiu V, Vail TP, et al. The epidemiology of revision total knee arthroplasty in the United States. Clinical Orthopaedics and Related Research 2010;468(1):45–51. 2010 Jan; Epub 2009/06/26.

11. Toulson C, Walcott-Sapp S, Hur J, Salvati E, Bostrom M, Brause B, et al. Treatment of infected total hip arthroplasty with a 2-stage reimplantation protocol: update on "our institution's" experience from 1989 to 2003. The Journal of Arthroplasty 2009;24(7):1051–60. 2009 Oct; Epub 2008/10/14.

12. Berend KR, Lombardi AV, Jr., Morris MJ, Bergeson AG, Adams JB, Sneller MA. Two-stage treatment of hip periprosthetic joint infection is associated with a high rate of infection control but high mortality. Clinical Orthopaedics and Related Research 2013;471(2):510–8. 2013 Feb; Epub 2012/09/18.

13. Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA. Periprosthetic joint infection. Lancet 2016;387(10016):386–94. 2016 Jan 23; Epub 2015/07/03.

14. Coventry MB. Treatment of infections occurring in total hip surgery. The Orthopedic Clinics of North America 1975;6(4):991–1003. 1975 Oct; Epub 1975/10/01.

15. Fitzgerald RH, Jr., Nolan DR, Ilstrup DM, Van Scoy RE, Washington JA, 2nd, Coventry MB. Deep wound sepsis following total hip arthroplasty. The Journal of Bone and Joint Surgery American Volume. 1977;59(7):847–55. 1977 Oct; Epub 1977/10/01.

16. Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. The Journal of Bone and Joint Surgery American Volume. 1996;78(4):512–23. 1996 Apr; Epub 1996/04/01.

17. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. The New England Journal of Medicine 2004;351(16):1645–54. 2004 Oct 14; Epub 2004/10/16.

18. Barrett L, Atkins B. The clinical presentation of prosthetic joint infection. The Journal of Antimicrobial Chemotherapy 2014;69 Suppl 1:i25–7. 2014 Sep; Epub 2014/08/20.

19. Zimmerli W. Infection and musculoskeletal conditions: Prosthetic-joint-associated infections. Best Practice & Research. Clinical Rheumatology 2006;20(6):1045–63. 2006 Dec; Epub 2006/11/28.

20. Izakovicova P, Borens O, Trampuz A. Periprosthetic joint infection: Current concepts and outlook. EFORT Open Reviews 2019;4(7):482–94. 2019 Jul; Epub 2019/08/20.

21. Romano CL, Romano D, Logoluso N, Drago L. Bone and joint infections in adults: A comprehensive classification proposal. European Orthopaedics and Traumatology 2011;1(6):207–17. 2011 May; Epub 2011/08/13.

22. McPherson EJ, Woodson C, Holtom P, Roidis N, Shufelt C, Patzakis M. Periprosthetic total hip infection: outcomes using a staging system. Clinical Orthopaedics and Related Research 2002(403):8–15. 2002 Oct; Epub 2002/10/03.

23. Cierny G, 3rd, Mader JT, Penninck JJ. A clinical staging system for adult osteomyelitis. Clinical Orthopaedics and Related Research. 2003(414):7–24. 2003 Sept; Epub 2003/09/11.

24. Cierny G, 3rd, DiPasquale D. Periprosthetic total joint infections: staging, treatment, and outcomes. Clinical Orthopaedics and Related Research. 2002 403:23–8. 2002 Oct; Epub 2002/10/03.

25. Southwood RT, Rice JL, McDonald PJ, Hakendorf PH, Rozenbilds MA. Infection in experimental arthroplasties. Clinical Orthopaedics and Related Research 1987(224):33–6. 1987 Nov; Epub 1987/11/01.

26. Southwood RT, Rice JL, McDonald PJ, Hakendorf PH, Rozenbilds MA. Infection in experimental hip arthroplasties. The Journal of Bone and Joint Surgery British Volume. 1985;67(2):229–31. 1985 Mar; Epub 1985/03/01.

27. Sendi P, Banderet F, Graber P, Zimmerli W. Periprosthetic joint infection following Staphylococcus aureus bacteremia. The Journal of Infection 2011;63(1):17–22. 2011 Jul; Epub 2011/06/15.

28. Zeller V, Kerroumi Y, Meyssonnier V, Heym B, Metten MA, Desplaces N, et al. Analysis of postoperative and hematogenous prosthetic joint-infection microbiological patterns in a large cohort. The Journal of Infection 2018;76(4):328–34. 2018 Apr; Epub 2018/02/06.

29. Lee J, Kang CI, Lee JH, Joung M, Moon S, Wi YM, et al. Risk factors for treatment failure in patients with prosthetic joint infections. The Journal of Hospital Infection 2010;75(4):273–6. 2010 Aug; Epub 2010/07/17.

30. Konigsberg BS, Della Valle CJ, Ting NT, Qiu F, Sporer SM. Acute hematogenous infection following total hip and knee arthroplasty. The Journal of Arthroplasty 2014;29(3):469–72. 2014 Mar; Epub 2013/09/04.

31. Rodriguez D, Pigrau C, Euba G, Cobo J, Garcia-Lechuz J, Palomino J, et al. Acute haematogenous prosthetic joint infection: prospective evaluation of medical and surgical management. Clinical Microbiology and Infection 2010;16(12):1789–95. 2010 Dec; Epub 2010/11/17.

32. Illingworth KD, Mihalko WM, Parvizi J, Sculco T, McArthur B, el Bitar Y, et al. How to minimize infection and thereby maximize patient outcomes in total joint arthroplasty: a multicenter approach: AAOS exhibit selection. The Journal of Bone and Joint Surgery American Volume. 2013;95(8):e50. 2013 Apr 17; Epub 2013/04/19.

33. Canale ST, Beaty JH. Campbell's operative orthopaedics London e-book: Elsevier Health Sciences; 2012.

34. Tan TL, Shohat N, Rondon AJ, Foltz C, Goswami K, Ryan SP, et al. Perioperative Antibiotic Prophylaxis in Total Joint Arthroplasty: A Single Dose Is as Effective as Multiple Doses. The Journal of Bone and Joint Surgery American Volume. 2019;101(5):429–37. 2019 Mar 6; Epub 2019/03/08.

35. Saeed K, McLaren AC, Schwarz EM, Antoci V, Arnold WV, Chen AF, et al. 2018 international consensus meeting on musculoskeletal infection: Summary from the biofilm workgroup and consensus on biofilm related musculoskeletal infections. Journal of Orthopaedic Research 2019;37(5):1007–17. 2019 May; Epub 2019/01/23.

36. Stewart PS. Antimicrobial Tolerance in Biofilms. Microbiol Spectrum. 2015;3(3). 2015 Jun; Epub 2015/07/18.

37. Cramton SE, Gerke C, Schnell NF, Nichols WW, Gotz F. The intercellular adhesion (ica) locus is present in Staphylococcus aureus and is required for biofilm formation. Infection and Immunity 1999;67(10):5427–33. 1999 Oct; Epub 1999/09/25.
38. Roy R, Tiwari M, Donelli G, Tiwari V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence 2018;9(1):522–54. 2018 Jan 1; Epub 2017/04/01.
39. Walters MC, 3rd, Roe F, Bugnicourt A, Franklin MJ, Stewart PS. Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of Pseudomonas aeruginosa biofilms to ciprofloxacin and tobramycin. Antimicrobial Agents and Chemotherapy 2003;47(1):317–23. 2003 Jan; Epub 2002/12/25.
40. Brown MR, Allison DG, Gilbert P. Resistance of bacterial biofilms to antibiotics: a growth-rate related effect? The Journal of Antimicrobial Chemotherapy 1988;22(6):777–80. 1988 Dec; Epub 1988/12/01.
41. Anderl JN, Zahller J, Roe F, Stewart PS. Role of nutrient limitation and stationary-phase existence in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. Antimicrobial Agents and Chemotherapy 2003;47(4):1251–6. 2003 Apr; Epub 2003/03/26.
42. Ma H, Bryers JD. Non-invasive determination of conjugative transfer of plasmids bearing antibiotic-resistance genes in biofilm-bound bacteria: effects of substrate loading and antibiotic selection. Applied Microbiology and Biotechnology 2013;97(1):317–28. 2013 Jan; Epub 2012/06/07.
43. Koseki H, Yonekura A, Shida T, Yoda I, Horiuchi H, Morinaga Y, et al. Early staphylococcal biofilm formation on solid orthopaedic implant materials: in vitro study. PLoS One 2014;9(10):e107588. Epub 2014/10/10.
44. Rochford ET, Richards RG, Moriarty TF. Influence of material on the development of device-associated infections. Clinical Microbiology and Infection 2012;18(12):1162–7. 2012 Dec; Epub 2012/08/29.
45. Otto M. Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity. Annual Review of Medicine 2013;64:175–88. Epub 2012/08/22.
46. Darouiche RO. Anti-infective efficacy of silver-coated medical prostheses. Clinical Infectious Diseases 1999;29(6):1371–7; quiz 8. 1999 Dec; Epub 1999/12/10.
47. Lusiak-Szelachowska M, Zaczek M, Weber-Dabrowska B, Miedzybrodzki R, Klak M, Fortuna W, et al. Phage neutralization by sera of patients receiving phage therapy. Viral Immunology 2014;27(6):295–304. 2014 Aug; Epub 2014/06/04.
48. Defoirdt T. Quorum-Sensing Systems as Targets for Antivirulence Therapy. Trends in Microbiology 2018;26(4):313–28. Epub 2017/11/15.
49. Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, et al. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. Clinical Orthopaedics and Related Research 2011;469(11):2992–4. Epub 2011/09/23.
50. Parvizi J, Gehrke T. Definition of periprosthetic joint infection. The Journal of Arthroplasty 2014;29(7):1331. Epub 2014/04/29.
51. Parvizi J, Tan TL, Goswami K, Higuera C, Della Valle C, Chen AF, et al. The 2018 definition of periprosthetic hip and knee infection: An evidence-based and validated criteria. The Journal of Arthroplasty. 2018;33(5):1309–14.e2. Epub 2018/03/20.
52. Signore A, Sconfienza LM, Borens O, Glaudemans A, Cassar-Pullicino V, Trampuz A, et al. Consensus document for the diagnosis of prosthetic joint infections: A joint paper by the EANM, EBJIS, and ESR (with ESCMID endorsement). European Journal of Nuclear Medicine and Molecular Imaging 2019;46(4):971–88. 2019 Apr; Epub 2019/01/27.
53. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Executive summary: diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clinical Infectious Diseases 2013;56(1):1–10. 2013 Jan; Epub 2012/12/12.

54. Lee YS, Koo KH, Kim HJ, Tian S, Kim TY, Maltenfort MG, et al. Synovial fluid biomarkers for the diagnosis of periprosthetic joint infection: A systematic review and meta-analysis. The Journal of Bone and Joint Surgery American Volume 2017;99(24):2077–84. 2017 Dec 20; Epub 2017/12/20.

55. Shahi A, Kheir MM, Tarabichi M, Hosseinzadeh HRS, Tan TL, Parvizi J. Serum D-Dimer Test is promising for the diagnosis of periprosthetic joint infection and timing of reimplantation. The Journal of Bone and Joint Surgery American Volume 2017;99(17):1419–27. 2017 Sep 6; Epub 2017/09/06.

56. Wyatt MC, Beswick AD, Kunutsor SK, Wilson MJ, Whitehouse MR, Blom AW. The alpha-defensin immunoassay and leukocyte esterase colorimetric strip test for the diagnosis of periprosthetic infection: A systematic review and meta-analysis. The Journal of Bone and Joint Surgery American Volume 2016;98(12):992–1000. 2016 Jun 15; Epub 2016/06/17.

57. Tischler EH, Cavanaugh PK, Parvizi J. Leukocyte esterase strip test: Matched for musculoskeletal infection society criteria. The Journal of Bone and Joint Surgery American Volume 2014;96(22):1917–20. 2014 Nov 19; Epub 2014/11/21.

58. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J. Combined measurement of synovial fluid alpha-Defensin and C-reactive protein levels: highly accurate for diagnosing periprosthetic joint infection. The Journal of Bone and Joint Surgery American Volume 2014;96(17):1439–45. 2014 Sep 3; Epub 2014/09/05.

59. Tarabichi M, Shohat N, Goswami K, Alvand A, Silibovsky R, Belden K, et al. Diagnosis of periprosthetic joint infection: The potential of next-generation sequencing. The Journal of Bone and Joint Surgery American Volume 2018;100(2):147–54. 2018 Jan 17; Epub 2018/01/18.

60. Shahi A, Tan TL, Kheir MM, Tan DD, Parvizi J. Diagnosing periprosthetic joint infection: And the winner is? The Journal of Arthroplasty 2017;32(9s):S232-s5. 2017 Sep; Epub 2017/07/18.

61. Omar M, Ettinger M, Reichling M, Petri M, Guenther D, Gehrke T, et al. Synovial C-reactive protein as a marker for chronic periprosthetic infection in total hip arthroplasty. The Bone & Joint Journal. 2015;97-b(2):173–6. 2015 Feb; Epub 2015/01/30.

62. Diagnosis and Prevention of Periprosthetic Joint Infections Clinical Practice Guideline. American Academy of Orthopaedic Surgeons. 2019.

63. Shohat N, Bauer T, Buttaro M, Budhiparama N, Cashman J, Della Valle CJ, et al. Hip and knee section, what is the definition of a periprosthetic joint infection (PJI) of the knee and the hip? Can the same criteria be used for both joints?: Proceedings of international consensus on orthopedic infections. The Journal of Arthroplasty 2019;34(2s):S325-s7. 2019 Feb; Epub 2018/10/23.

64. Renz N, Yermak K, Perka C, Trampuz A. Alpha defensin lateral flow test for diagnosis of periprosthetic joint infection: Not a screening but a confirmatory test. The Journal of Bone and Joint Surgery American Volume 2018;100(9):742–50. 2018 May 2; Epub 2018/05/02.

65. Ricciardi BF, Muthukrishnan G, Masters EA, Kaplan N, Daiss JL, Schwarz EM. New developments and future challenges in prevention, diagnosis, and treatment of prosthetic joint infection. Journal of Orthopaedic Research. 2020. 2020 Jan 22; Epub 2020/01/23.

66. Berbari E, Mabry T, Tsaras G, Spangehl M, Erwin PJ, Murad MH, et al. Inflammatory blood laboratory levels as markers of prosthetic joint infection: A systematic review and meta-analysis. The Journal of Bone and Joint Surgery American Volume 2010;92(11):2102–9. 2010 Sep 1; Epub 2010/09/03.

67. Austin MS, Ghanem E, Joshi A, Lindsay A, Parvizi J. A simple, cost-effective screening protocol to rule out periprosthetic infection. The Journal of Arthroplasty 2008;23(1):65–8. 2008 Jan; Epub 2008/01/01.

68. Alijanipour P, Bakhshi H, Parvizi J. Diagnosis of periprosthetic joint infection: The threshold for serological markers. Clinical Orthopaedics and Related Research 2013;471(10):3186–95. 2013 Oct; Epub 2013/05/22.

69. Bounameaux H, de Moerloose P, Perrier A, Reber G. Plasma measurement of D-dimer as diagnostic aid in suspected venous thromboembolism: an overview. Thrombosis and Haemostasis 1994;71(1):1–6. 1994 Jan; Epub 1994/01/01.

70. Gris JC, Bouvier S, Cochery-Nouvellon E, Faillie JL, Lissalde-Lavigne G, Lefrant JY. Fibrin-related markers in patients with septic shock: individual comparison of D-dimers and fibrin monomers impacts on prognosis. Thrombosis and Haemostasis 2011;106(6):1228–30. 2011 Dec; Epub 2011/09/29.

71. Schwameis M, Steiner MM, Schoergenhofer C, Lagler H, Buchtele N, Jilma-Stohlawetz P, et al. D-dimer and histamine in early stage bacteremia: A prospective controlled cohort study. European Journal of Internal Medicine 2015;26(10):782–6. 2015 Dec; Epub 2015/11/21.

72. Qin L, Li F, Gong X, Wang J, Huang W, Hu N. Combined measurement of D-dimer and C-reactive protein levels: Highly accurate for diagnosing chronic periprosthetic joint infection. The Journal of Arthroplasty 2020;35(1):229–34. 2020 Jan; Epub 2019/09/19.

73. Chandy S, Joseph K, Sankaranarayanan A, Issac A, Babu G, Wilson B, et al. Evaluation of C-reactive protein and fibrinogen in patients with chronic and aggressive periodontitis: A clinico-biochemical study. Journal of Clinical and Diagnostic Research. 2017;11(3):Zc41-zc5. 2017 Mar; Epub 2017/05/18.

74. Li R, Shao HY, Hao LB, Yu BZ, Qu PF, Zhou YX, et al. Plasma fibrinogen exhibits better performance than plasma D-dimer in the diagnosis of periprosthetic joint infection: A multicenter retrospective study. The Journal of Bone and Joint Surgery American Volume. 2019;101(7):613–9. 2019 Apr 3; Epub 2019/04/05.

75. Pieper CF, Rao KM, Currie MS, Harris TB, Cohen HJ. Age, functional status, and racial differences in plasma D-dimer levels in community-dwelling elderly persons. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 2000;55(11):M649–57. 2000 Nov; Epub 2000/11/15.

76. Bottner F, Wegner A, Winkelmann W, Becker K, Erren M, Gotze C. Interleukin-6, procalcitonin and TNF-alpha: markers of peri-prosthetic infection following total joint replacement. The Journal of Bone and Joint Surgery American Volume 2007;89(1):94–9. 2007 Jan; Epub 2007/01/30.

77. Yoon JR, Ko YR, Shin YS. Effect of shape on bone cement polymerization time in knee joint replacement surgery. Medicine (Baltimore) 2018;97(17):e0558. 2018 Apr; Epub 2018/04/29.

78. Wirtz DC, Heller KD, Miltner O, Zilkens KW, Wolff JM. Interleukin-6: a potential inflammatory marker after total joint replacement. International Orthopaedics 2000;24(4):194–6. Epub 2000/11/18.

79. Selberg O, Hecker H, Martin M, Klos A, Bautsch W, Kohl J. Discrimination of sepsis and systemic inflammatory response syndrome by determination of circulating plasma concentrations of procalcitonin, protein complement 3a, and interleukin-6. Critical Care Medicine 2000;28(8):2793–8. 2000 Aug; Epub 2000/08/31.

80. Ahmad SS, Hirschmann MT, Becker R, Shaker A, Ateschrang A, Keel MJB, et al. A meta-analysis of synovial biomarkers in periprosthetic joint infection: Synovasure is less effective than the ELISA-based alpha-defensin test. Knee Surgery, Sports Traumatology, Arthroscopy: Official Journal of the ESSKA. 2018;26(10):3039–47. 2018 Oct; Epub 2018/03/21.

81. Xie K, Dai K, Qu X, Yan M. Serum and Synovial Fluid Interleukin-6 for the Diagnosis of Periprosthetic Joint Infection. Scientific Reports 2017;7(1):1496. 2017 May 4; Epub 2017/05/06.

82. Randau TM, Friedrich MJ, Wimmer MD, Reichert B, Kuberra D, Stoffel-Wagner B, et al. Interleukin-6 in serum and in synovial fluid enhances the differentiation between periprosthetic joint infection and aseptic loosening. PLoS One 2014;9(2):e89045. Epub 2014/03/04.

83. Dinneen A, Guyot A, Clements J, Bradley N. Synovial fluid white cell and differential count in the diagnosis or exclusion of prosthetic joint infection. The Bone & Joint Journal. 2013;95-b(4):554–7. 2013 Apr; Epub 2013/03/30.

84. Zmistowski B, Restrepo C, Huang R, Hozack WJ, Parvizi J. Periprosthetic joint infection diagnosis: a complete understanding of white blood cell count and differential. The Journal of Arthroplasty 2012;27(9):1589–93. 2012 Oct; Epub 2012/05/01.

85. Bedair H, Ting N, Jacovides C, Saxena A, Moric M, Parvizi J, et al. The Mark Coventry Award: Diagnosis of early postoperative TKA infection using synovial fluid analysis. Clinical Orthopaedics and Related Research 2011;469(1):34–40. 2011 Jan; Epub 2010/06/30.

86. Ghanem E, Parvizi J, Burnett RS, Sharkey PF, Keshavarzi N, Aggarwal A, et al. Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. The Journal of Bone and Joint Surgery American Volume 2008;90(8):1637–43. 2008 Aug; Epub 2008/08/05.

87. Proceedings of the International Consensus Meeting on Periprosthetic Joint Infection. Foreword. Journal of Orthopaedic Research. 2014;32 Suppl 1:S2–3. 2014 Jan; Epub 2014/01/28.

88. Goswami K, Parvizi J, Maxwell Courtney P. Current recommendations for the diagnosis of acute and chronic PJI for hip and knee-cell counts, alpha-defensin, leukocyte esterase, Next-generation Sequencing, Current Reviews in Musculoskeletal Medicine 2018;11(3):428–38. 2018 Sep; Epub 2018/08/01.

89. Schinsky MF, Della Valle CJ, Sporer SM, Paprosky WG. Perioperative testing for joint infection in patients undergoing revision total hip arthroplasty. The Journal of Bone and Joint Surgery American Volume. 2008;90(9):1869–75. 2008 Sep; Epub 2008/09/03.

90. Shahi A, Deirmengian C, Higuera C, Chen A, Restrepo C, Zmistowski B, et al. Premature therapeutic antimicrobial treatments can compromise the diagnosis of late periprosthetic joint infection. Clinical Orthopaedics and Related Research 2015;473(7):2244–9. 2015 Jul; Epub 2015/01/22.

91. Ghanem E, Houssock C, Pulido L, Han S, Jaberi FM, Parvizi J. Determining "true" leukocytosis in bloody joint aspiration. The Journal of Arthroplasty 2008;23(2):182–7. 2008 Feb; Epub 2008/02/19.

92. Shahi A, Parvizi J, Kazarian GS, Higuera C, Frangiamore S, Bingham J, et al. The alpha-defensin test for periprosthetic joint infections is not affected by prior antibiotic administration. Clinical Orthopaedics and Related Research 2016;474(7):1610–5. 2016 Jul; Epub 2016/02/13.

93. Cipriano CA, Brown NM, Michael AM, Moric M, Sporer SM, Della Valle CJ. Serum and synovial fluid analysis for diagnosing chronic periprosthetic infection in patients with inflammatory arthritis. The Journal of Bone and Joint Surgery American Volume. 2012;94(7):594–600. 2012 Apr 4; Epub 2012/04/11.

94. Parvizi J, Jacovides C, Adeli B, Jung KA, Hozack WJ., Mark B. Coventry Award: synovial C-reactive protein: a prospective evaluation of a molecular marker for periprosthetic knee joint infection. Clinical Orthopaedics and Related Research 2012;470(1):54–60. 2012 Jan; Epub 2011/07/26.

95. Tetreault MW, Wetters NG, Moric M, Gross CE, Della Valle CJ. Is synovial C-reactive protein a useful marker for periprosthetic joint infection? Clinical Orthopaedics and Related Research 2014;472(12):3997–4003. 2014 Dec; Epub 2014/07/30.

96. Stone WZ, Gray CF, Parvataneni HK, Al-Rashid M, Vlasak RG, Horodyski M, et al. Clinical evaluation of synovial alpha defensin and synovial C-reactive protein in the diagnosis of periprosthetic joint infection. The Journal of Bone and Joint Surgery American Volume. 2018;100(14):1184–90. 2018 Jul 18; Epub 2018/07/19.

97. Deirmengian C, Kardos K, Kilmartin P, Gulati S, Citrano P, Booth RE, Jr. The alpha-defensin test for periprosthetic joint infection responds to a wide spectrum of organisms. Clinical Orthopaedics and Related Research 2015;473(7):2229–35. 2015 Jul; Epub 2015/01/30.

98. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J. Diagnosing periprosthetic joint infection: has the era of the biomarker arrived? Clinical Orthopaedics and Related Research 2014;472(11):3254–62. 2014 Nov; Epub 2014/03/05.

99. Kleiss S, Jandl NM, Novo de Oliveira A, Ruther W, Niemeier A. Diagnostic accuracy of alpha-defensin enzyme-linked immunosorbent assay in the clinical evaluation of painful hip and knee arthroplasty with possible prosthetic joint infection: a prospective study of 202 cases. The Bone & Joint Journal. 2019;101-b(8):970–7. 2019 Aug; Epub 2019/08/01.

100. Bonanzinga T, Zahar A, Dutsch M, Lausmann C, Kendoff D, Gehrke T. How reliable is the alpha-defensin immunoassay test for diagnosing periprosthetic joint infection? A prospective study. Clinical Orthopaedics and Related Research 2017;475(2):408–15. 2017 Feb; Epub 2016/06/28.

101. Sigmund IK, Yermak K, Perka C, Trampuz A, Renz N. Is the enzyme-linked immunosorbent assay more accurate than the lateral flow alpha defensin test for diagnosing periprosthetic joint infection? Clinical Orthopaedics and Related Research 2018;476(8):1645–54. 2018 Aug; Epub 2018/07/19.

102. Bonanzinga T, Ferrari MC, Tanzi G, Vandenbulcke F, Zahar A, Marcacci M. The role of alpha defensin in prosthetic joint infection (PJI) diagnosis: A literature review. EFORT Open Rev. 2019;4(1):10–3. 2019 Jan; Epub 2019/02/26.

103. Zagra L, Villa F, Cappelletti L, Gallazzi E, Materazzi G, De Vecchi E. Can leucocyte esterase replace frozen sections in the intraoperative diagnosis of prosthetic hip infection? The Bone & Joint Journal. 2019;101-b(4):372–7. 2019 Apr; Epub 2019/04/02.

104. Wetters NG, Berend KR, Lombardi AV, Morris MJ, Tucker TL, Della Valle CJ. Leukocyte esterase reagent strips for the rapid diagnosis of periprosthetic joint infection. The Journal of Arthroplasty 2012;27(8 Suppl):8–11. 2012 Sep; Epub 2012/05/23.

105. Qu X, Zhai Z, Wu C, Jin F, Li H, Wang L, et al. Preoperative aspiration culture for preoperative diagnosis of infection in total hip or knee arthroplasty. Journal of Clinical Microbiology 2013;51(11):3830–4. 2013 Nov; Epub 2013/08/16.

106. Hughes JG, Vetter EA, Patel R, Schleck CD, Harmsen S, Turgeant LT, et al. Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. Journal of Clinical Microbiology 2001;39(12):4468–71. 2001 Dec; Epub 2001/11/29.

107. Font-Vizcarra L, Garcia S, Martinez-Pastor JC, Sierra JM, Soriano A. Blood culture flasks for culturing synovial fluid in prosthetic joint infections. Clinical Orthopaedics and Related Research 2010;468(8):2238–43. 2010 Aug; Epub 2010/02/18.

108. Geller JA, MacCallum KP, Murtaugh TS, Patrick DA, Jr., Liabaud B, Jonna VK. Prospective comparison of blood culture bottles and conventional swabs for microbial identification of suspected periprosthetic joint infection. The Journal of Arthroplasty 2016;31(8):1779–83. 2016 Aug; Epub 2016/03/30.

109. Peel TN, Spelman T, Dylla BL, Hughes JG, Greenwood-Quaintance KE, Cheng AC, et al. Optimal periprosthetic tissue specimen number for diagnosis of prosthetic joint infection. Journal of Clinical Microbiology 2017;55(1):234–43. 2017 Jan; Epub 2016/11/04.

110. Bemer P, Leger J, Tande D, Plouzeau C, Valentin AS, Jolivet-Gougeon A, et al. How many samples and how many culture media to diagnose a prosthetic joint infection: A clinical and microbiological prospective multicenter study. Journal of Clinical Microbiology 2016;54(2):385–91. 2016 Feb; Epub 2015/12/08.

111. Tsaras G, Maduka-Ezeh A, Inwards CY, Mabry T, Erwin PJ, Murad MH, et al. Utility of intraoperative frozen section histopathology in the diagnosis of periprosthetic joint infection: a systematic review and meta-analysis. The Journal of Bone and Joint Surgery American Volume 2012;94(18):1700–11. 2012 Sep 19; Epub 2012/09/21.

112. Inagaki Y, Uchihara Y, Munemoto M, Scarborough M, Dodd CAF, Gibbons C, et al. Correlation of histological and microbiological findings in septic and aseptic knee implant failure. Archives of Orthopaedic and Trauma Surgery 2019;139(5):717–22. 2019 May; Epub 2019/03/13.

113. Sadiq S, Wootton JR, Morris CA, Northmore-Ball MD. Application of core biopsy in revision arthroplasty for deep infection. The Journal of Arthroplasty 2005;20(2):196–201. 2005 Feb; Epub 2005/05/21.

114. Bemer P, Plouzeau C, Tande D, Leger J, Giraudeau B, Valentin AS, et al. Evaluation of 16S rRNA gene PCR sensitivity and specificity for diagnosis of prosthetic joint infection: A prospective multicenter cross-sectional study. Journal of Clinical Microbiology 2014;52(10):3583–9. 2014 Oct; Epub 2014/07/25.

115. Liu K, Fu J, Yu B, Sun W, Chen J, Hao L. Meta-analysis of sonication prosthetic fluid PCR for diagnosing periprosthetic joint infection. PLoS One 2018;13(4):e0196418. Epub 2018/04/28.

116. Tarabichi M, Alvand A, Shohat N, Goswami K, Parvizi J. Diagnosis of Streptococcus canis periprosthetic joint infection: the utility of next-generation sequencing. Arthroplast Today. 2018;4(1):20–3. 2018 Mar; Epub 2018/03/22.

117. Thoendel M, Jeraldo P, Greenwood-Quaintance KE, Chia N, Abdel MP, Steckelberg JM, et al. A novel prosthetic joint infection pathogen, mycoplasma salivarium, identified by metagenomic shotgun sequencing. Clinical Infectious Diseases 2017;65(2):332–5.

118. Wang C, Huang Z, Li W, Fang X, Zhang W. Can metagenomic next-generation sequencing identify the pathogens responsible for culture-negative prosthetic joint infection? BMC Infectious Diseases 2020;20(1):253. 2020 Mar 30; Epub 2020/04/02.

119. Carli AV, Abdelbary H, Ahmadzai N, Cheng W, Shea B, Hutton B, et al. Diagnostic accuracy of serum, synovial, and tissue testing for chronic periprosthetic joint infection after hip and knee replacements: A systematic review. The Journal of Bone and Joint Surgery American Volume 2019;101(7):635–49. 2019 Apr 3; Epub 2019/04/05.

120. Di Cesare PE, Chang E, Preston CF, Liu CJ. Serum interleukin-6 as a marker of periprosthetic infection following total hip and knee arthroplasty. The Journal of Bone and Joint Surgery American Volume 2005;87(9):1921–7. 2005 Sep; Epub 2005/09/06.

121. Buttaro MA, Tanoira I, Comba F, Piccaluga F. Combining C-reactive protein and interleukin-6 may be useful to detect periprosthetic hip infection. Clinical Orthopaedics and Related Research 2010;468(12):3263–7. 2010 Dec; Epub 2010/07/14.

122. Worthington T, Dunlop D, Casey A, Lambert R, Luscombe J, Elliott T. Serum procalcitonin, interleukin-6, soluble intercellular adhesin molecule-1 and IgG to short-chain exocellular lipoteichoic acid as predictors of infection in total joint prosthesis revision. British Journal of Biomedical Science 2010;67(2):71–6. Epub 2010/07/31.

123. Abou El-Khier NT, El Ganainy Ael R, Elgeidy A, Rakha SA. Assessment of interleukin-6 and other inflammatory markers in the diagnosis of Egyptian patients with periprosthetic joint infection. The Egyptian Journal of Immunology 2013;20(2):93–9. Epub 2013/01/01.

124. Glehr M, Friesenbichler J, Hofmann G, Bernhardt GA, Zacherl M, Avian A, et al. Novel biomarkers to detect infection in revision hip and knee arthroplasties. Clinical Orthopaedics and Related Research 2013;471(8):2621–8. 2013 Aug; Epub 2013/04/24.

125. Gollwitzer H, Dombrowski Y, Prodinger PM, Peric M, Summer B, Hapfelmeier A, et al. Antimicrobial peptides and proinflammatory cytokines in periprosthetic joint infection. The Journal of Bone and Joint Surgery American Volume. 2013;95(7):644–51. 2013 Apr 3; Epub 2013/04/05.

126. Ettinger M, Calliess T, Kielstein JT, Sibai J, Bruckner T, Lichtinghagen R, et al. Circulating biomarkers for discrimination between aseptic joint failure, low-grade infection, and high-grade septic failure. Clinical Infectious Diseases 2015;61(3):332–41. 2015 Aug 1; Epub 2015/04/15.

127. Gallo J, Svoboda M, Zapletalova J, Proskova J, Juranova J. Serum IL-6 in combination with synovial IL-6/CRP shows excellent diagnostic power to detect hip and knee prosthetic joint infection. PLoS One 2018;13(6):e0199226. Epub 2018/06/22.

128. Deirmengian C, Hallab N, Tarabishy A, Della Valle C, Jacobs JJ, Lonner J, et al. Synovial fluid biomarkers for periprosthetic infection. Clinical Orthopaedics and Related Research 2010;468(8):2017–23. 2010 Aug; Epub 2010/03/20.

129. Frangiamore SJ, Gajewski ND, Saleh A, Farias-Kovac M, Barsoum WK, Higuera CA. Alpha-defensin accuracy to diagnose periprosthetic joint infection-best available test? The Journal of Arthroplasty 2016;31(2):456–60. 2016 Feb; Epub 2015/11/08.

130. Jacovides CL, Parvizi J, Adeli B, Jung KA. Molecular markers for diagnosis of periprosthetic joint infection. The Journal of Arthroplasty. 2011;26(6 Suppl):99–103.e1. 2011 Sep; Epub 2011/05/17.
131. Lenski M, Scherer MA. The significance of interleukin-6 and lactate in the synovial fluid for diagnosing native septic arthritis. Acta Orthopaedica Belgica 2014;80(1):18–25. 2014 Mar; Epub 2014/05/31.
132. Nilsdotter-Augustinsson A, Briheim G, Herder A, Ljunghusen O, Wahlstrom O, Ohman L. Inflammatory response in 85 patients with loosened hip prostheses: a prospective study comparing inflammatory markers in patients with aseptic and septic prosthetic loosening. Acta Orthopaedica 2007;78(5):629–39. 2007 Oct; Epub 2007/10/30.
133. Buttaro MA, Martorell G, Quinteros M, Comba F, Zanotti G, Piccaluga F. Intraoperative synovial C-reactive protein is as useful as frozen section to detect periprosthetic hip infection. Clinical Orthopaedics and Related Research 2015;473(12):3876–81. 2015 Dec; Epub 2015/05/28.
134. Parvizi J, McKenzie JC, Cashman JP. Diagnosis of periprosthetic joint infection using synovial C-reactive protein. The Journal of Arthroplasty 2012;27(8 Suppl):12–6. 2012 Sep; Epub 2012/05/09.
135. Ronde-Oustau C, Diesinger Y, Jenny JY, Antoni M, Gaudias J, Boeri C, et al. Diagnostic accuracy of intra-articular C-reactive protein assay in periprosthetic knee joint infection – A preliminary study. Orthopaedics & Traumatology, Surgery & Research : OTSR. 2014;100(2):217–20. 2014 Apr; Epub 2014/03/04.
136. Vanderstappen C, Verhoeven N, Stuyck J, Bellemans J. Intra-articular versus serum C-reactive protein analysis in suspected periprosthetic knee joint infection. Acta Orthopaedica Belgica 2013;79(3):326–30. 2013 Jun; Epub 2013/08/10.
137. De Vecchi E, Villa F, Bortolin M, Toscano M, Tacchini L, Romano CL, et al. Leucocyte esterase, glucose and C-reactive protein in the diagnosis of prosthetic joint infections: a prospective study. Clinical Microbiology and Infection 2016;22(6):555–60. 2016 Jun; Epub 2016/04/05.
138. De Vecchi E, Romano CL, De Grandi R, Cappelletti L, Villa F, Drago L. Alpha defensin, leukocyte esterase, C-reactive protein, and leukocyte count in synovial fluid for pre-operative diagnosis of periprosthetic infection. International Journal of Immunopathology and Pharmacology 2018;32:2058738418806072. 2018 Mar–Dec; Epub 2018/11/01.
139. Kim SG, Kim JG, Jang KM, Han SB, Lim HC, Bae JH. Diagnostic value of synovial white blood cell count and serum C-reactive protein for acute periprosthetic joint infection after knee arthroplasty. The Journal of Arthroplasty 2017;32(12):3724–8. 2017 Dec; Epub 2017/08/13.
140. Sousa R, Serrano P, Gomes Dias J, Oliveira JC, Oliveira A. Improving the accuracy of synovial fluid analysis in the diagnosis of prosthetic joint infection with simple and inexpensive biomarkers: C-reactive protein and adenosine deaminase. The Bone & Joint Journal. 2017;99-b(3):351–7. 2017 Mar; Epub 2017/03/03.
141. Plate A, Anagnostopoulos A, Glanzmann J, Stadler L, Weigelt L, Sutter R, et al. Synovial C-reactive protein features high negative predictive value but is not useful as a single diagnostic parameter in suspected periprosthetic joint infection (PJI). The Journal of Infection 2019;78(6):439–44. 2019 Jun; Epub 2019/04/10.
142. Bingham J, Clarke H, Spangehl M, Schwartz A, Beauchamp C, Goldberg B. The alpha defensin-1 biomarker assay can be used to evaluate the potentially infected total joint arthroplasty. Clinical Orthopaedics and Related Research 2014;472(12):4006–9. 2014 Dec; Epub 2014/09/27.
143. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Booth RE, Jr., et al. The alpha-defensin test for periprosthetic joint infection outperforms the leukocyte esterase test strip. Clinical Orthopaedics and Related Research 2015;473(1):198–203. 2015 Jan; Epub 2014/06/20.

144. Kasparek MF, Kasparek M, Boettner F, Faschingbauer M, Hahne J, Dominkus M. Intraoperative diagnosis of periprosthetic joint infection using a novel alpha-defensin lateral flow assay. The Journal of Arthroplasty 2016;31(12):2871–4. 2016 Dec; Epub 2016/06/23.

145. Sigmund IK, Holinka J, Gamper J, Staats K, Bohler C, Kubista B, et al. Qualitative alpha-defensin test (Synovasure) for the diagnosis of periprosthetic infection in revision total joint arthroplasty. The Bone & Joint Journal. 2017;99-b(1):66–72. 2017 Jan; Epub 2017/01/06.

146. Suda AJ, Tinelli M, Beisemann ND, Weil Y, Khoury A, Bischel OE. Diagnosis of periprosthetic joint infection using alpha-defensin test or multiplex-PCR: Ideal diagnostic test still not found. International Orthopaedics 2017;41(7):1307–13. 2017 Jul; Epub 2017/02/06.

147. Berger P, Van Cauter M, Driesen R, Neyt J, Cornu O, Bellemans J. Diagnosis of prosthetic joint infection with alpha-defensin using a lateral flow device: a multicentre study. The Bone & Joint Journal. 2017;99-b(9):1176–82. 2017 Sep; Epub 2017/09/02.

148. Balato G, Franceschini V, Ascione T, Lamberti A, D'Amato M, Ensini A, et al. High performance of alpha-defensin lateral flow assay (Synovasure) in the diagnosis of chronic knee prosthetic infections. Knee Surgery, Sports Traumatology, Arthroscopy : Official Journal of the ESSKA. 2018;26(6):1717–22. 2018 Jun; Epub 2017/10/11.

149. Gehrke T, Lausmann C, Citak M, Bonanzinga T, Frommelt L, Zahar A. The accuracy of the alpha defensin lateral flow device for diagnosis of periprosthetic joint infection: Comparison with a gold standard. The Journal of Bone and Joint Surgery American Volume 2018;100(1):42–8. 2018 Jan 3; Epub 2018/01/04.

150. Sigmund IK, Holinka J, Lang S, Stenicka K, Staats K, Hobusch G, et al. A comparative study of intraoperative frozen section and alpha defensin lateral flow test in the diagnosis of periprosthetic joint infection. Acta Orthopaedica 2019;90(2):105–10. 2019 Apr; Epub 2019/01/24.

151. Riccio G, Cavagnaro L, Akkouche W, Carrega G, Felli L, Burastero G. Qualitative alpha-defensin versus the main available tests for the diagnosis of periprosthetic joint infection: Best predictor test? Journal of Bone and Joint Infection 2018;3(3):156–64. Epub 2018/08/22.

152. de Saint Vincent B, Migaud H, Senneville E, Loiez C, Pasquier G, Girard J, et al. Diagnostic accuracy of the alpha defensin lateral flow device (Synovasure) for periprosthetic infections in microbiologically complex situations: A study of 42 cases in a French referral centre. Orthopaedics & Traumatology, Surgery & Research : OTSR. 2018;104(4):427–31. 2018 Jun; Epub 2018/03/28.

153. Tahta M, Simsek ME, Isik C, Akkaya M, Gursoy S, Bozkurt M. Does inflammatory joint diseases affect the accuracy of infection biomarkers in patients with periprosthetic joint infections? A prospective comparative reliability study. Journal of Orthopaedic Science : Official Journal of the Japanese Orthopaedic Association 2019;24(2):286–9. 2019 Mar; Epub 2018/10/01.

154. Plate A, Stadler L, Sutter R, Anagnostopoulos A, Frustaci D, Zbinden R, et al. Inflammatory disorders mimicking periprosthetic joint infections may result in false-positive alpha-defensin. Clinical Microbiology and Infection. 2018;24(11):1212.e1-1212.e6. 2018 Nov; Epub 2018/03/03.

155. Hischebeth GT, Randau TM, Buhr JK, Wimmer MD, Hoerauf A, Molitor E, et al. Unyvero i60 implant and tissue infection (ITI) multiplex PCR system in diagnosing periprosthetic joint infection. Journal of Microbiological Methods 2016;121:27–32. 2016 Feb; Epub 2015/12/23.

156. Prieto-Borja L, Rodriguez-Sevilla G, Aunon A, Perez-Jorge C, Sandoval E, Garcia-Canete J, et al. Evaluation of a commercial multiplex PCR (Unyvero i60((R))) designed for the diagnosis of bone and joint infections using prosthetic-joint sonication. Enfermedades Infecciosas y Microbiología Clínica 2017;35(4):236–42. 2017 Apr; Epub 2016/10/23.

157. Lausmann C, Zahar A, Citak M, Branes J, Schmidl S, Frommelt L, et al. Are there benefits in early diagnosis of prosthetic joint infection with multiplex polymerase chain reaction? Journal of Bone and Joint Infection 2017;2(4):175–83. Epub 2017/11/10.

158. Portillo ME, Salvado M, Sorli L, Alier A, Martinez S, Trampuz A, et al. Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure. The Journal of Infection 2012;65(6):541–8. 2012 Dec; Epub 2012/09/11.
159. Suren C, Feihl S, Cabric S, Banke IJ, Haller B, Trampuz A, et al. Improved pre-operative diagnostic accuracy for low-grade prosthetic joint infections using second-generation multiplex Polymerase chain reaction on joint fluid aspirate. International Orthopaedics. 2020. 2020 Apr 15; Epub 2020/04/17.
160. Morgenstern C, Cabric S, Perka C, Trampuz A, Renz N. Synovial fluid multiplex PCR is superior to culture for detection of low-virulent pathogens causing periprosthetic joint infection. Diagnostic Microbiology and Infectious Disease 2018;90(2):115–9. 2018 Feb; Epub 2017/12/02.

Chapter 5
Soft Tissue Infections

Rajendra Sawh-Martinez and Sabrina N. Pavri

Abstract Infections of the skin, subcutaneous fat, muscle, and connective tissues include cellulitis, soft tissue abscesses, tenosynovitis, myositis and necrotizing fasciitis. This chapter aims to provide a broad overview of the presentation of these clinical entities in a progressive manner, with a special focus on the management, reconstruction and novel therapies aimed at ameliorating the ravages of soft tissue infections. Despite the significant promise of an emerging panoply of naturally occurring and synthetic materials aimed at improving wound healing, new technologies and advances face the challenges of completing randomized controlled clinical trials to demonstrate efficacy in wound healing. As such, mainstay principles of controlling comorbidities (diabetes, vascular disease, nicotine avoidance, nutrition, *etc.*) and wound management principles (debridement, infectious control, surgical management of abscesses/necrosis) remain the keystone of wound care.

Keywords Soft tissue · Infection · Cellulitis · Abscess · Tenosynovitis · Myositis · Wounds · Management · Necrotizing fasciitis · Wound care · Novel therapies

5.1 Overview

Infections of the skin, subcutaneous fat, muscle, and connective tissues (fascia) that envelop our deeper structures encompass a wide spectrum of clinical entities thought of as "soft tissue infections." These tissues surround and envelop our skeleton and

R. Sawh-Martinez (✉)
AdventHealth for Children, Orlando, FL, USA

Biionix Cluster Faculty, University of Central Florida College of Medicine, Orlando, FL, USA

S. N. Pavri
University of Central Florida College of Medicine, Orlando, FL, USA

Aesthetic and Reconstructive Surgery Institute, Orlando Health Cancer Institute, Orlando, FL, USA

may all be prone to the ravaging effects of contaminants (viral and bacterial) with a wide spectrum of presentation from the mild to the life threatening.

This chapter aims to provide a broad overview of the presentation of these clinical entities in a progressive manner, with a special focus on the management, reconstruction, and novel therapies aimed at ameliorating the ravages of soft tissue infections.

5.2 Cellulitis

Cellulitis, from the Latin cellula (diminutive of cella: cell) and "itis" (a suffix denoting inflammation), is defined as a diffuse bacterial infection of the skin and underlying subcutaneous tissue. It is characterized by a well-demarcated, superficially spreading area of erythema with irregular borders, typically unilateral, and without an underlying collection of purulent fluid (abscess). There is typically a causal event involving entry of bacteria through the skin barrier, although this may or may not have been noticed by the patient. As a non-reportable condition, the precise incidence remains unknown; however, it is one of the most common reasons for urgent care/emergency department (ED) visits and acute hospitalization, leading to significant morbidity and cost to the healthcare system.

5.2.1 A Diagnostic Challenge?

The traditionally taught characteristics of cellulitis, "rubor, tumor, calor, dolor" (erythema, swelling, heat, and pain), are neither sensitive nor specific, and a broad differential must often be entertained when considering the diagnosis as a wide range of conditions share a similar clinical presentation. A recent study reported that almost one-third of patients hospitalized with a diagnosis of cellulitis are found to be misdiagnosed, leading to an estimated 50,000–130,000 unnecessary hospitalizations and $195 million–$515 million in avoidable healthcare spending annually in the United States [1]. Conditions frequently misdiagnosed as cellulitis include stasis dermatitis, deep vein thrombosis, thrombophlebitis, gout, lymphedema, hematoma, and contact dermatitis (Table 5.1) [2].

Cellulitis remains a predominantly clinical diagnosis, and while adjuncts such as labs and imaging can often help to identify an alternative diagnosis, there is no single sensitive and specific test for cellulitis. The history remains a crucial part of diagnosis, with key points including the onset, pattern, and speed of symptom progression; age and medical comorbidities (diabetes, chronic kidney or liver disease, heart failure, vascular disease, malignancy, and immunosuppression); recent antimicrobial treatment; history of previous cellulitis; travel history; animal or human bites; exposure to salt or freshwater (including pools/spas); exposure to animals, fish, or reptiles; and history of IV drug use. Lab abnormalities seen may include a

Table 5.1 Causes of pseudocellulitis and how to differentiate them

Stasis dermatitis	Bilateral in nature, typically on the lower extremities between the knees and ankles, gradual symptom onset, red to brown hyperpigmentation, history of peripheral vascular disease/insufficiency
Gout	Focal warmth, erythema, tenderness, and edema typically limited to a single joint (usually the knee or first metatarsal-phalangeal joint), history of gout, tophi
Deep vein thrombosis	History of recent trauma, surgery, immobilization, or cancer; thrombosis on ultrasound
Contact dermatitis	Erythema limited to areas in contact with irritant, may be pruritic
Thrombophlebitis	Inflammation of superficial veins, often with palpable, tender, erythematous cords
Lymphedema	Edema of the extremities (unilateral > bilateral), positive Kaposi-Stemmer's sign
Hematoma	Red to purplish discoloration of the skin, firm or fluctuant subcutaneous mass depending on time course, often with a history of trauma/anticoagulation
Necrotizing fasciitis	Pain out of proportion to clinical findings, rapid onset, systemic illness, bullae, purple or blue discoloration of the skin, cutaneous crepitation

Fig. 5.1 Cellulitis of the dorsal hand with lymphangitis (erythematous streaking along dermal lymphatics)

leukocytosis with a left shift indicating bacterial infection (seen in 35–50% of patients), and an elevated CRP (C-reactive protein), seen in 60–95% of patients [3]. Fever and systemic signs and symptoms of infection are not typically seen as the infection is localized to a specific area of soft tissue (Fig. 5.1). Testing modalities used to diagnose other infections such as culture swabs are not helpful, as a skin swab over an area of cellulitis will merely grow the normal bacteria colonizing the skin and not the cause of the deeper infection. Tissue culture using a punch biopsy of the skin is usually considered an excessively invasive procedure as it further creates a break in the skin's protective barrier, and final culture and bacterial sensitivity results take several days – too long to be helpful in guiding antibiotic treatment of mild to moderate cases. As such, tissue culture is usually limited to the rare cases

where an uncommon infectious agent is suspected to be present (typically when exposure history is positive for animal bites or water exposure), or in cases resistant to broad-spectrum empiric treatment. According to recommendations published by the Infectious Diseases Society of America (IDSA), blood cultures are not routinely recommended unless the patient has a malignancy on chemotherapy, neutropenia, severe cell-mediated immunodeficiency, or a history of immersion injuries, or animal bites [4]. However, these recommendations are rarely followed, and data suggest that approximately a third of patients presenting with cellulitis receive blood cultures (only 10% of which are indicated according to guidelines), and over two-thirds of patients received at least one modality of imaging (almost all of which is contraindicated by the IDSA guidelines and which changed management in less than 10% of cases) – leading to an estimated $226.9 million dollars annually spent on largely clinically useless diagnostic studies [5]. Ultrasound is a fast, cost-effective, and widely available modality helpful to assess for complicating factors such as underlying fluid collections (abscesses or hematomas), vascular thromboses, or the presence of foreign bodies. Findings of cellulitis on ultrasound include increased echogenicity and thickness of the skin and a "cobblestoning pattern" caused by anechoic strands intersected by inflamed subcutaneous fat, both of which indicate nonspecific tissue edema. If CT or MRI is used, findings include thickening of the skin and underlying fascia and infiltration of subcutaneous fat [6].

5.2.2   Risk Factors

As the body's largest organ, the skin has innate immunoprotective mechanisms that are compromised in trauma, old age, and a variety of comorbid chronic conditions such as diabetes, vascular disease, and obesity. In a prospective study involving over 600 patients admitted with cellulitis, 54.8% had wounds that are predisposed to the development of cellulitis (most commonly skin ulcers in 18.2% and nonsurgical trauma in 17.8%). A quarter of the patients reported a previous episode of cellulitis, and among other risk factors, diabetes was reported in 25.2% of the patients, venous insufficiency in 20.5%, edema or lymphedema in 27.7%, obesity in 37.8%, immunosuppression in 11.6%, and diverse other comorbidities in 74.6% (Fig. 5.2) [7].

5.2.3   Etiology

The causative organism in cellulitis is identified in only about one-quarter of cases due to the diffuse nature of the infection; however when isolated, *Staphylococcus aureus* and *Streptococcus* species are the most common [7]. A history of a human or animal bite wound in the cellulitic area should raise concerns for atypical organisms, most commonly *Pasteurella multocida* (cats), *Eikenella corrodens* (dogs), and *Streptococcus viridans* (human bites). Exposure to fresh or salt water must raise
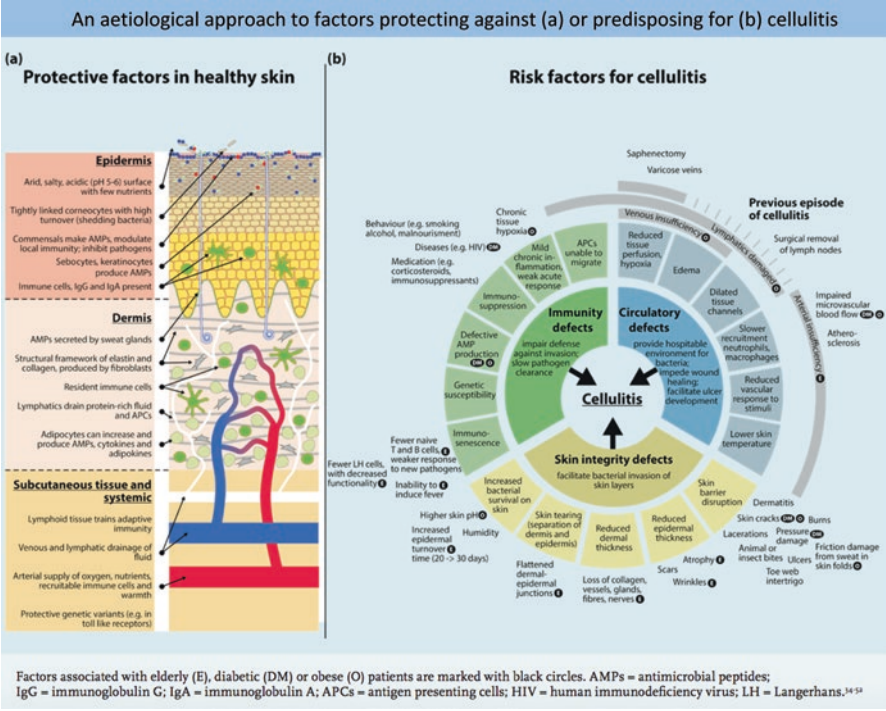
Fig. 5.2 Local and systemic factors predisposing to and protecting against cellulitis [8]

concerns for atypical mycobacterial infections (*Mycobacterium marinum*) and *Vibrio vulnificus*, and envenomation from aquatic animal species should be ruled out (Table 5.2) [9].

5.2.4   Treatment

According to guidelines updated in 2014 by the IDSA [4], mild cases of cellulitis (without systemic symptoms or a purulent focus) should receive a 5-day course of an oral agent effective against *Streptococci*, although most clinicians will also include coverage for *Staph. aureus*. Options for treatment include cephalexin, clindamycin, or amoxicillin-clavulanate. Serological studies suggest that group A *Streptococcus* is the most common cause of culture-negative cellulitis, while cellulitis with purulence is strongly associated with *Staph. aureus* infection [10].

   For patients whose cellulitis is associated with penetrating trauma, evidence of methicillin-resistant *S. aureus* (MRSA) infection elsewhere, nasal colonization with MRSA, and injection drug use, an antimicrobial effective against both MRSA and *streptococci* is recommended (doxycycline, clindamycin, trimethoprim-sulfamethoxazole).

Table 5.2 Atypical pathogens in cellulitis

Condition	Possible atypical pathogens
Neutropenia	*Exherichia coli* Enterobacteriaceae *Pseudomonas aeruginosa*
Liver cirrhosis	*E. coli, Klebsiella spp, Pseudomonas spp, Proteus spp, Aeromonas spp, Vibrio spp, Acinetobacter spp*
Diabetic foot infection	
• Chronic ulcer, or ulcer previously treated with antibiotics	Enterobacteriaceae
• Macerated ulcer	*P. aeruginosa* (in combination with other organisms)
• Long duration nonhealing wounds with Prolonged, broad-spectrum antibiotic treatment	Enterococci, diphtheroids, Enterobacteriaciae, Pseudomonas spp, nonfermentative gram-negative rods
Fresh or salt water exposure	*Aeromonas hydrophila, Edwardsiella tarda, Erysipelothrix rhusipathiae, Mycobacterium fortuitum, Mycobacterium marinum, Shewanella putrefaciens, Streptococcus iniae*
• Tropical/warm water	*Chromobacterium violaceum, Vibro vulnificus*
Fish fin or bone injuries	*Enterobacter spp, Erysipelothrix rhusiopathiae, Klebsiella pneumoniae, Mycobacteria marium, Streptococcus iniae, Vibrio vulnificus*
Human bites	*Eikenella corrodens, Haemophilus spp, Enterobacteriaceae, Gemella morbillorum, Neisseria spp, Prevotella spp, Fusobacterium spp, Eubacterium spp, Veillonella spp, Peptostreptococcus spp*
Cat or dog bites	*Pasteurella spp, Neisseria spp, Corynebacterium spp, Moraxella spp, Enterococcus spp, Fusobacterium spp, Porphyromonas spp, Prevotella spp, Propionibacterium spp, Bacteriodes spp, Peptostreptococcus spp*

Cranendonk DR, Lavrijsen APM, Prins JM, Wiersinga WJ. Cellulitis: current insights into pathophysiology and clinical management. Neth J Med. 2017;75(9):366–378

Antibiotics recommended for MRSA infections include [11]:

Oral options:
• Minocycline 100 mg q12h
Trimethoprim and sulfamethoxazole 160/800 mg q12h
Doxycycline 100 mg q12h
Clindamycin 300–600 mg q8h (high resistance rate
Linezolid 600 mg q12h
Tedizolid 200 mg q24 h

Intravenous options:
• Vancomycin 15 mg/kg IV q12h
Teicoplanin LD 12 mg/kg IV q12h for three doses and then 6 mg/kg q12h
Tigecycline 100 mg IV as a single dose, then 50 mg IV q12h
Linezolid 600 mg q12h
Daptomycin 4–6 mg/kg q24h
Ceftaroline 600 mg q12h
Dalbavancin 1000 mg once followed by 500 mg after 1 week or 1500 mg one dose
Tedizolid 200 mg q24h

For patients with systemic symptoms, IV (rather than PO) therapy is recommended, and in high-risk patients (malignancy on chemotherapy, neutropenia, severe cell-mediated immunodeficiency, immersion injuries, and animal bites), IV vancomycin plus either piperacillin-tazobactam or imipenem/meropenem is

recommended as an empiric regimen. Duration of antibiotic therapy recommended is typically 5 days, although this can be extended if the infection has not improved within this time frame (Fig. 5.3). However, without noted improvement one must harbor suspicions for an underlying abscess, atypical pathogen, or other complicating factor. For patients started on IV antibiotic therapy, conversion to oral therapy is usually undertaken after approximately 1–3 days, provided there is clinical improvement.

Outpatient therapy is recommended for patients who do not have systemic inflammatory response syndrome (SIRS), altered mental status, or hemodynamic instability. Inpatient hospitalization is recommended if there is concern for a deeper or necrotizing infection, for patients with poor adherence to therapy, for infection in a severely immunocompromised patient, or if outpatient treatment is failing. Risk factors for poor outcomes include three or more episodes of prior cellulitis, lower rates of nonsurgical trauma, and presence of venous insufficiency, immunosuppression, and sepsis [7].

Fig. 5.3 Improvement of dorsal hand cellulitis after 48 h of IV antibiotics, immobilization (splinting), and elevation

5.3   Soft Tissue Abscess

A soft tissue abscess is a walled-off collection of infected fluid (pus) within the skin and subcutaneous tissue. Soft tissue abscesses often develop and are seen in conjunction with a cellulitis, and an untreated or undertreated cellulitis may often progress to an abscess as the body attempts to wall off the bacteria. While *Streptococcus* species are more commonly responsible for non-purulent cellulitis, *Staph. aureus* (either methicillin-sensitive (MSSA) or MRSA) is typically seen in purulent cellulitis and abscesses.

Clinical evaluation is often enough to diagnose a soft tissue abscess, with characteristics of a fluctuant subcutaneous mass, and (if superficial enough) discoloration/thinning of the overlying skin, occasionally with purulent drainage through the skin surface. However, when the clinical picture is uncertain, ultrasound is a low-cost imaging modality widely available at the point of care that is approximately 91% sensitive, 77% specific, and changes management in 10% of cases when it comes to differentiating soft tissue abscesses from cellulitis [12].

The primary treatment for a soft tissue abscess is incision and drainage. This must be performed in such a way that:

1. The purulent fluid collection is completely evacuated, with all loculations broken up.
2. The purulent fluid must be sent for Gram stain and aerobic and anaerobic culture.
3. Any purulent fluid that could recollect has a way to drain.

As such, needle aspiration of an abscess is not an appropriate treatment as it does not allow persistent ongoing drainage and the abscess is almost guaranteed to recur. The dead space left after the incision and drainage of an abscess heals by secondary intention, which refers to healing of an open wound from the base upwards, by granulation, contraction, and epithelialization. There are multiple techniques for incision and drainage, the choice of which is often dictated by the location, size, and depth of the abscess, the patient's socioeconomic situation and willingness/ability to perform wound care, and the healthcare provider's familiarity with the various techniques.

If the abscess is very superficial, often the overlying skin will be discolored (white or purple) and devitalized. In these cases, it is best to debride the devitalized skin and allow the open wound to heal. In deeper abscesses, the subdermal vascular plexus maintains the viability of the skin, and if after an incision and drainage the skin edges are allowed to come into apposition, they will heal faster than the abscess cavity fills in by secondary intention, and the abscess will recollect. Because of this, when linear incisions are made, they should extend the length of the fluid collection, and packing of the wound should be performed daily to prevent fluid accumulation and wound edge apposition. Cruciate incisions can be performed instead, but are significantly more morbid in the amount of scarring created.

The choice of packing material can vary, but needs to be absorbent and not able to break down in the wound, thereby leaving foreign particulate matter behind.

Typically, saline-moistened gauze is recommended, but common packing materials also include plain or iodoform-impregnated cotton strips, or a hydrofiber like Aquacel (sodium carboxymethylcellulose).

An alternative technique called the loop drainage technique (LDT) was compared to conventional incision and drainage (CID) and demonstrated a lower abscess recollection rate (4.1% versus 9.4%). This technique is primarily used in the pediatric patient population where compliance with packing is often limited due to the patient's age, but should be considered in the adult population for deep abscesses given the potential for decreased pain/scarring, fewer follow-up visits, and lower healthcare costs [13]. The LDT involves making a small incision at each end of the abscess, performing blunt dissection to break down loculations, and inserting a vessel loop through the incisions that is tied on the skin surface. This allows for continued drainage of the abscess cavity with a smaller incision and no need to repack the wound.

While the primary treatment of soft tissue abscesses consists of incision and drainage, a meta-analysis of 4 randomized placebo-controlled trials involving over 2000 participants found that concurrent antibiotic treatment with drugs that cover MRSA (clindamycin or trimethoprim-sulfamethoxazole) was associated with a significantly increased primary lesion cure rate (risk reduction of 7.4%) and reduced new lesion development rate (10%), with a slightly increased rate of minor adverse events (4.4%) [14].

5.4   Tenosynovitis and Myositis

Myositis and tenosynovitis represent two distinct clinical entities along the spectrum of progressing depth of invasion of bacterial contamination. We will treat these separately for the purposes of this chapter, but clinically they may present simultaneously or in series with evolving disease burden [15].

5.4.1   Tenosynovitis

Tendons are strong, fibrous connective tissues that are ropelike extensions of muscles which generally attach to bones. In the skeletal system, tendons act as connective bands and pulleys that allow for complex translation of muscle contractions. Tendons are covered by a peritenon layer and often pass through synovial sheaths which are osseofibrous tunnels that reduce friction between tendons and the surrounding structures. Tenosynovitis refers to inflammation of the tendon and its synovial sheath. There are three main mechanisms by which this infection may occur, and they are all associated with bacterial contamination. Most commonly a direct trauma with inoculation of a large bacterial burden leads to the inflammatory reaction. Infections of the synovial sheaths may also occur from either

hematogenous spread (least common) or contiguous spread from adjacent compromised soft tissues. Although this can occur and affect any tendon, it is most associated with hand and wrist involvement and can severely affect tendon gliding and mobility. When found in the lower extremity tendons, the infection limits the patient's ability to bear weight [16, 17].

Classically, tenosynovitis occurs on the flexor tendons of the hand. In this setting, there are five cardinal clinical signs of infection, often referred to as Kanavel signs. These include fusiform enlargement of the affected digit (most common), finger held in flexion, tenderness along the course of the flexor sheath, and pain along the tendon with passive extension (earliest) [18, 19]. Like most infections, there is a progression from initial exposure and mild irritation to widespread and tissue destruction. Severe disease states are often encountered as the initial inciting events in tenosynovitis are mild and sometimes unrecognized. Bites from animals or slight punctures can deliver high bacterial loads into deep spaces with little appreciation for the potential sequela. Intermittent swelling or symptoms may be mild or incompletely addressed which allows for a deep space infection along the tendon sheath to promulgate.

Based on the anatomy of the tendon sheaths and wrist, these infections may progress into surrounding deep spaces leading to involvement of separate tendons via contiguous bursa or connections in the hand and wrist. For example, a " horseshoe abscess" may form when an infection in either the thumb or small finger spreads into the radial or ulnar bursa, respectively, which are often connected in the wrist via the space of Parona. Similar spread may occur from the extensor tendons on the dorsum of the hand to the flexor tendons on the palmar surface. Proximal spreading along deep tissue planes may also affect the contiguous carpal tunnel and forearm [20, 21]. This further spread if left untreated may lead to compartment syndrome, tissue necrosis, and hematogenous spread.

The stages of progression in tenosynovitis include [18, 22]:

Stage 1: Accumulation of exudative fluid and distention of the potential space in the tendon sheath
Stage 2: Purulent fluid accumulation and distention of the tendon sheath
Stage 3: Necrosis and destruction of the tendon sheath, surrounding retinacular support structures (Fig. 5.4)

A full review of all the potentially involved pathogens is beyond the scope of this section; however, the most involved pathogens are listed below [23, 24].

Most common pathogens in tenosynovitis:

- *Staphylococcus aureus*
- *Streptococcus* spp.
- *Pasteurella multocida*
- *Eikenella corrodens*
- *Mycobacterium marinum*

The timing and severity of each stage can vary based on the inciting pathogen and the degree of bacterial burden. Generally, infections due to *Staphylococcus*

aureus or group A *Streptococcus* present within days, whereas infections due to *Pasteurella multocida* or *Mycobacterium marinum* may present within days or weeks of the original infection. In settings of bites, lacerations, and diabetes, polymicrobial infections, including gram-negative organisms, are most common including combinations of the above noted pathogens [24]. Punctures or thorn-based injuries may also lead to fungal tenosynovitis.

Fig. 5.4 (**a**) Stage 1: Accumulation of exudative fluid and distention of the potential space in the tendon sheath. (**b**) Stage 2: Purulent fluid accumulation and distention of the tendon sheath. (**c**) Stage 3: Necrosis and destruction of the tendon sheath, surrounding retinacular support structures. All include the Kanavel signs which include fusiform swelling of affected digit, finger held in flexion, tenderness along course of the flexor sheath, and pain along the tendon with passive extension

A wide variety of clinical entities may mimic tenosynovitis, and distinguishing between them is critical for timely intervention.

Differential diagnosis:
- Gout
- Herpes zoster
- Psoriatic arthritis
- Rheumatoid arthritis (RA)
- Pseudogout
- de Quervain stenosing tenosynovitis
- Trigger finger
- Trauma
- Soft tissue infection

Early diagnosis is imperative and establishing the infectious source key to help guide management. Understanding and evaluating for the Kanavel signs aids in diagnosis, and the need for intervention in tenosynovitis should be suspected in patients with findings resembling soft tissue infection that do not improve with antimicrobial therapy. Definitive diagnosis may be established by tendon sheath aspiration, with fluid culture (including bacteria, mycobacteria, and fungal culture) and histopathologic examination. Although diagnostic imaging cannot evaluate for infection, plain radiographs may evaluate for bony involvement and demonstrate opaque foreign bodies, the degree of soft tissue swelling, and potential radiographic changes consistent with osteomyelitis. While ultrasound, CT scan, and MRI are potentially useful adjuncts to define the anatomical involvement, they are not routinely needed as the diagnosis of tenosynovitis should be based on the clinical constellation of symptoms.

Early management of infections suspicious for tenosynovitis can vary based on the presentation; however, once the diagnosis of tenosynovitis is made, the mainstay of treatment is IV antibiotics and surgical release of the deep space infection (Sect. 5.6). Early treatment of infections consists of oral broad-spectrum antibiotics targeting the presumed inciting microbe (skin flora, oral flora, fungal). Common presentations of tenosynovitis have failed prior attempts of oral antibiotics, which present with worsening symptoms while on antibiotics or acutely with fusiform swelling and pain with lack of treatment. Patients with delayed presentations may also have lymphatic or hematogenous spread, and a sepsis workup and/or blood cultures should be considered. Tenosynovitis may also occur *from* hematogenous spread and is associated with *N. gonorrhoeae* and Mycobacteria.

IV antibiotics should be started immediately upon suspicion of tenosynovitis, and a timely surgical evaluation is needed to treat the acute infection to avoid devastating complications from stage 3 disease (necrosis and destruction of the tendon sheath). Patients with mild symptoms that are being managed conservatively should also have their extremity elevated, in a protective splint with restricted activity. If tenosynovitis is suspected, and the clinical infection does not clear with antibiotics, it should be presumed that the tendon sheath is involved, and early surgical exploration is warranted. The tendon sheath should be irrigated and drained, with

appropriate debridement of necrotic tissue if needed. Classically, the tendon sheath is irrigated continuously postoperatively to ensure adequate clearance of infectious material, often with need for repeat surgical exploration and wound irrigation.

Given the closed space infection, if there isn't early, adequate treatment tenosynovitis can lead to significant destruction of the tendon sheath and surrounding support structures, potentially leading to irrevocable damage in terms of finger range of motion and function. Finger stiffness, tendon adhesions/scars, tendon necrosis, and boutonniere deformity may all occur in cases with delayed treatment or stage 3 presentation and may even require amputation [25].

5.4.2 Myositis

Infections of skeletal muscle may lead to significant swelling and necrosis of affected muscles. Myositis is a broad term that encompasses various clinical entities that result in inflammation of muscles. These may include dermatomyositis, polymyositis, necrotizing myopathy, and inclusion body myositis. Myositis in the setting of infectious myositis or necrotizing myopathy as a clinical entity may have common symptoms including fever, malaise, and muscle pain.

Differentiating between mild forms of myositis can be challenging, and muscle biopsies are the mainstay for diagnosis. Imaging modalities such as MRI may also help elucidate the extent of affected swelling and localized infections. In the setting of soft tissue infections, the involvement of deeper structures is usually progressive, and overlying skin infections, drug injections, infected insect bites, and diabetic patients should raise suspicion for muscle involvement. Myositis may be caused by any infectious agent, including viruses (HIV), mycobacteria, fungi, and parasites.

Common infectious causes of myositis:
• *Staphylococcus aureus* (psoas abscess)
Streptococcus groups A, B, C, and G
Enterobacteriaceae
Yersinia enterocolitica
Pseudomonas spp.
Aeromonas spp.
Clostridium spp. (especially *perfringens*)
Peptostreptococcus spp.
Bacteroides spp.

Contiguous spread from skin/subcutaneous abscess, penetrating wounds, osteomyelitis, trauma, and pressure ulcers are the most common causes of clinical myositis [26]. A primary muscle abscess can occur in the absence of surrounding infection and may occur from hematogenous spread and/or in the setting of vascular insufficiency [27]. Historically, myositis has been thought of as a "tropical" infection from infectious agents such as filariasis and malaria in settings of predisposition from diabetes, steroid therapy, or immunosuppressive states. However, myositis

may result from involvement of muscle in infections in any setting with uncontrolled, spreading infections and is the natural next step of tissue compromise in a variety of settings.

Similarly to soft tissue abscesses, diagnosis and early treatment are cornerstones of treatment to avoid progression of involved tissues and systematic effects. In the absence of obvious surrounding infection, muscle biopsy may be indicated when there is high clinical suspicion or imaging identified muscle involvement to evaluate for idiopathic inflammatory myopathy. These clinical entities are often derived from a variety of autoimmune or rheumatic diseases and are listed below. A complete discussion of each of these entities is beyond the scope of this chapter, but these are important entities to be aware of in the differential diagnosis of myositis.

Idiopathic inflammatory myopathies:
• Dermatomyositis
Polymyositis
Myositis of the antisynthetase syndrome
Immune-mediated necrotizing myopathy
Inclusion body myositis
Systemic lupus erythematosus
Systemic sclerosis/scleroderma
Mixed connective tissue disease (MCTD)
Rheumatoid arthritis
Sjögren's syndrome

Treatments of infections involving muscle tissue are critical for they portend progressive spread into critical structures and deep tissue necrosis. Wound debridement, drainage of infection, and clearance of necrotic tissue are critical to allow for improvement of muscle infection and prevent spread (Sect. 5.6). Differentiating between localized infection and spreading fascial involvement is a clinical diagnosis that differentiates between a potentially life-threatening infection (necrotizing fasciitis – Section 5.5). Laboratory markers such as creatinine kinase and lactate acid dehydrogenase are useful markers to track the degree and progress of infection with surgical intervention. Increasing levels may mark progression of infection or unidentified muscle necrosis/involvement. It is important to remember that creatinine kinase levels do not correlate to disease severity.

The diagnosis and clinical evaluation of myositis should coincide with an evaluation for compartment syndrome as the deep space swelling and inflammation may have significant deleterious effect on overall function. Compartment syndrome is a clinical diagnosis involving "the five Ps" which include pain, poikilothermia, paresthesias, paralysis, and pulselessness. Measurement of compartment pressure should also reveal elevated pressures (> 30 mmHg) that indicate compression of the muscular blood supply and compromise of associated neurovascular structures. The presence of compartment syndrome represents a surgical emergency and often goes along with or leads to myositis. Emergent fascial release should be performed to preserve function and limit progression of functional compromise of severe myositis.

5.5   Necrotizing Fasciitis (RSM)

Necrotizing fasciitis is a life-threatening form of soft tissue infections which can encompass necrotizing myositis and cellulitis [28, 29]. Early, aggressive intervention is critical when a necrotizing infection is suspected and is key to improve mortality and limit morbidity. Necrotizing infections are characterized by rampant tissue destruction with systemic signs of sepsis/toxicity including destabilization of vital signs (fever, low blood pressure, tachycardia, tachypnea).

Although the term "necrotizing fasciitis" refers to infection of a specific anatomic structure, tissue destruction may occur at several levels simultaneously, including the skin (cellulitis), soft tissue, muscle (myositis), and bone (osteomyelitis). Necrotizing fasciitis occurs predominantly in the fascial layer and includes several key distinct features which indicate a deep space, spreading infection involving the presence of gas in the tissues (crepitus on physical exam). It is often presumed that necrotizing fasciitis involves surrounding structures, making the specific diagnosis (i.e., necrotizing fasciitis vs. necrotizing myositis) somewhat indistinguishable on clinical exam.

Key features that produce life-threatening infectious spread include the limited blood supply of muscle fascia and the longitudinal structure of the anatomy which allows for rapid spread along tissues [28–30]. Isolated deep space infections which progress to necrotizing infections can often progress without full appreciation of the extent of infection as overlying tissue can appear unaffected, with only pain and underlying swelling as the early signs. Delayed presentation and diagnosis are notable causes of increased morbidity, and the high mortality is associated with necrotizing fasciitis (Fig. 5.5).

Necrotizing fasciitis category and common causes [29, 31–33]:

Type I – polymicrobial (anaerobic and aerobic bacteria)
- Anaerobic bacteria (at least one):
- *Bacteroides fragilis*, *Clostridium difficile*, and *Peptostreptococcus* sp.
- Aerobic:

     Escherichia coli, *Enterobacter*, *Klebsiella*, and *Proteus*

- Facultative anaerobic other than group A *Streptococcus*
- Rarely *Pseudomonas aeruginosa* (obligate aerobe) and fungi (candida)
- Clinical entities: Fournier's gangrene and head and neck

Type II – Monomicrobial
- Group A *Streptococci* (GAS) w/ M protein:
- M protein types 1 and 3 associated w/ *streptococcal* toxic shock syndrome
- Beta-hemolytic *streptococci*
- *Staphylococcus aureus*
- Unknown sources – hematogenous translocation (GAS)
- *Less commonly (water-based trauma)*: *Vibrio vulnificus* and *Aeromonas hydrophila*

Fig. 5.5 Sequelae of necrotizing infections. (**a**) Fournier's gangrene after removal of infected tissues down to healthy, bleeding tissue. (**b**) Lower extremity necrotizing infection requiring full-thickness debridement of involved tissues. (**c**) Amputation of nonviable upper extremity

Type II, monomicrobial infections with group A *Strep* are most associated with toxic shock syndrome due to the production of pyrogenic exotoxins. These proteins are associated with tissue destruction, shock, and organ failure [34].

Most necrotizing infections commonly involve the lower extremities given their involvement in common comorbidities including diabetic neuropathy and peripheral vascular disease. This follows these sites of common infections secondary to decreased blood flow and loss of protective sensation. Critically, necrotizing infections often present acutely with severe, progressive symptoms that worsen in hours. This critical clinical hallmark is a cornerstone of the high morbidity and mortality of necrotizing infections that spread quickly, prior to an appreciation for the full extent of clinical involvement. Rapid progression of disease and delayed presentations lead to progressive systemic toxicity, potential limb loss, and death, making early recognition of necrotizing infections critical to patient outcomes.

Unfortunately, laboratory abnormalities are nonspecific, but are useful clinical markers. Inflammatory and metabolic serum markers are often elevated, and may add detail to the physical exam and history. Elevation of serum creatinine, lactic acid, aspartate aminotransferase (AST) are suggestive of deep infections as opposed to cellulitis. Although blood cultures are often positive (~ 60% in type II necrotizing fasciitis), their utility is limited in polymicrobial disease. Imaging modalities may also demonstrate air and abscess collection in subcutaneous pockets, with tracking inflammation. As surgical exploration and debridement are critical, they should not be delayed when there is high clinical suspicion of a

necrotizing infection. Diagnosis is confirmed during surgical exploration with the identification of swollen, dull-gray appearance with exudate and tracking infection along tissue planes.

Mortality of necrotizing infections:
- Type I necrotizing fasciitis – 20%
- Type II necrotizing fasciitis – up to 30%
- Fournier's gangrene – up to 40%

5.6   Wound Management and Reconstruction

Wounds have multiple etiologies, including surgery, trauma, radiation, infection, and chronic conditions such as diabetes and vascular disease. Considerations and techniques for wound management vary according to the type of wound, and are outside the scope of this text. As such, this section will review wound management and reconstruction of wounds caused specifically by soft tissue infections. The primary objective in wound management is debridement of any infected or devitalized tissue to obtain a clean wound. Control of the debrided wound is obtained by avoiding gross contamination and starting local antiseptic wound care, and optimizing the overall health and nutritional status of the patient. After these steps have been taken, reconstructive techniques will be dictated by the size and location of the wound, as well as any exposed structures.

In order for a wound to heal, it must be free of infection, necrotic tissue, and any foreign material. Surgical debridement remains the gold standard to obtain this goal. Surgical principles of adequate debridement include wide opening of the affected area, evacuation/removal of all purulent and necrotic tissue (with cultures sent to microbiology as appropriate), excision back to healthy bleeding tissue, and copious irrigation to dilute any remaining contamination. In severe necrotizing soft tissue infections, often multiple debridements are necessary, as the ultimate extent of the damage may not be immediately evident at the initial procedure.

Special considerations must be taken when wounds are at high likelihood of having a biofilm, which is defined as a surface-attached, structured microbial community containing sessile bacterial cells embedded in a self-produced matrix of extracellular polymeric substances (EPS). A biofilm has the ability to concentrate environmental nutrients in its extracellular matrix, facilitates resistance to antimicrobial factors, and allows slow bacterial dispersion into the wound by detachment, supplying a persistent bacterial source population leading to chronic infection [35]. Mechanical disruption of a suspected biofilm during surgical wound debridement can be undertaken using a variety of techniques, including curettage of the cavity, or painting the cavity with methylene blue followed by excision of all colored tissue. Repopulation of a biofilm within 24 hours of debridement is common, so if definitive closure of a wound is planned, it should be appropriately timed with debridement [36]. Antibiotics with a high bioavailability should be used in conjunction

with mechanical debridement to appropriately treat biofilm-related infections. Oral options include clindamycin, rifampin, fluoroquinolones, and trimethoprim-sulfamethoxazole, all of which have bioavailability comparable to parenteral antibiotic therapy.

Another important factor in obtaining control over a wound secondary to soft tissue infection is prevention of continued wound contamination. For wounds in the perineal region (commonly secondary to Fournier's gangrene), one must consider fecal and/or urinary diversion as needed. While this can be done through nonsurgical means such as a Foley catheter or rectal tube, patients will often require more durable long-term diversion through means of a suprapubic catheter or diverting ostomy.

Antiseptic local wound care is also critical for maintaining a clean wound bed to optimize healing. Available topical antimicrobials for reducing bioburden and surface contamination include Dakin's solution (0.5% sodium hypochlorite), poly-hexamethylene biguanide (PHMB)/betaine (Prontosan), povidone-iodine, acetic acid, mafenide acetate, and various silver-containing dressings. Full-strength Dakin's solution can be significantly cytotoxic, and we recommend diluting it to a concentration of 0.025% (1:20 Dakin's solution diluted in sterile water or saline). For the initial stages of wound control when still dealing with infection/exudate, packing material will often be soaked in these antiseptic solutions, while silver dressings and negative pressure wound therapy (NPWT) may be used during the later stages as the wound becomes cleaner and dressing frequency decreases [35].

The patient's overall health must also be optimized for wound healing to be successful. Caloric needs increase during the wound healing process and are estimated at 30–35 kcal/kg, but may vary based on age, medical comorbidities, BMI, stage of the healing process, and the severity, size, and number of wounds [37]. Lab values such as prealbumin and albumin levels are often tracked over time to ensure adequate nutrition, with albumin being a general reflection of the patient's nutritional status over the preceding 3 months, and prealbumin being an indication of the patient's more recent nutritional status over the preceding 2–3 weeks. The patient's medical comorbidities also play an important role in optimizing wound healing, such as glucose control in diabetics, cessation of all nicotine-containing products, and ensuring optimal blood flow by correction of peripheral arterial disease.

Negative pressure wound therapy (NPWT), otherwise known as wound vacuum-assisted closure (VAC), is another adjunct that can be used to manage and decrease the size of extensive wounds. The technology involves the controlled application of constant or intermittent subatmospheric pressure to the local wound environment, using a sealed foam dressing connected to a vacuum pump. NPWT enhances wound healing by removing excess extracellular fluid and decreasing tissue edema, leading to increased vascularity and stabilization of the wound environment. It also reduces systemic and local mediators of inflammation in experimental models, and decreases matrix metalloproteinase activity and bacterial burden clinically. NPWT has been shown to increase fibroblast proliferation and migration, collagen organization, and the expression of vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2), thereby enhancing wound healing [38].

Surgical options for reconstruction of a wound are myriad and will depend on the wound size and location, as well as any critical exposed structures (vessels, tendon, nerves, bone, intra-abdominal contents, etc.) and the overall clinical status of the patient. The reconstructive ladder is a concept from the field of plastic surgery that teaches a stepwise approach to treating wounds, progressing from the most basic to the most complex. This concept is now considered to be outdated, with the "reconstructive elevator" as a more appropriate analogy, as a patient may be best served by a more complex option for their initial attempt at reconstruction (Fig. 5.6).

Wounds secondary to soft tissue infections are often limited to the subcutaneous tissue (without exposure of vital nerves/vessels or bone), and therefore can typically be left to heal by secondary intention or can be skin grafted to speed up the healing process in an extensive wound. Integra Bilayer Matrix Wound Dressing is a dermal substitute comprised of a porous matrix of cross-linked bovine tendon collagen and glycosaminoglycan, with an overlying semipermeable polysiloxane (silicone) layer. It can be applied over a clean, well-vascularized wound bed and will revascularize over 3–4 weeks, thereby forming a neodermis which can be re-epithelialized with a thin split thickness skin graft. This increases the durability of the graft and overall reconstruction, and reduces the depth of the wound and the thickness of the skin graft needed, and is especially useful over high-mobility areas where wound contractures and extensive scar tissue would be problematic (Figs. 5.7 and 5.8).

Occasionally a severe necrotizing soft tissue infection will leave exposed major blood vessels or nerves, tendon without paratenon, or bone without periosteum. In such situations a plastic surgeon is needed to consider either a local or pedicled flap or a microvascular free tissue transfer to obtain wound coverage, although these cases remain a small percentage of overall wounds from soft tissue infections.

In the case detailed below, the patient presented with a necrotizing fasciitis of the left neck secondary to a locally advanced perforated esophageal cancer. Debridement resulted in ligation of the left common carotid artery and internal jugular vein and exposure of those large vessel stumps as well as the vagus nerve. A plastic surgery consult for wound coverage was obtained due to the exposure of vital structure (and risk of vessel rupture from prolonged exposure), as well as due to the need to obtain expedient wound closure so the patient could progress to chemoradiation therapy for his newly diagnosed cancer. He underwent wound coverage with a left pedicled pectoralis muscle flap and split thickness skin graft and went on to heal uneventfully (Fig. 5.9). Other examples are shown in Figs. 5.10 and 5.11.

5.7 Novel Therapies for Wound Care

In 2019, the wound care market was a 19.8 billion dollar industry, and is projected to reach 24.8B in 2024. Management and adjunctive therapies for problematic and nonhealing wounds run the gamut from specialized dressings, particulate derived from embryologic sources, and experimental, novel therapeutics aimed at improving tissue regeneration while ameliorating deleterious effects from comorbidities.
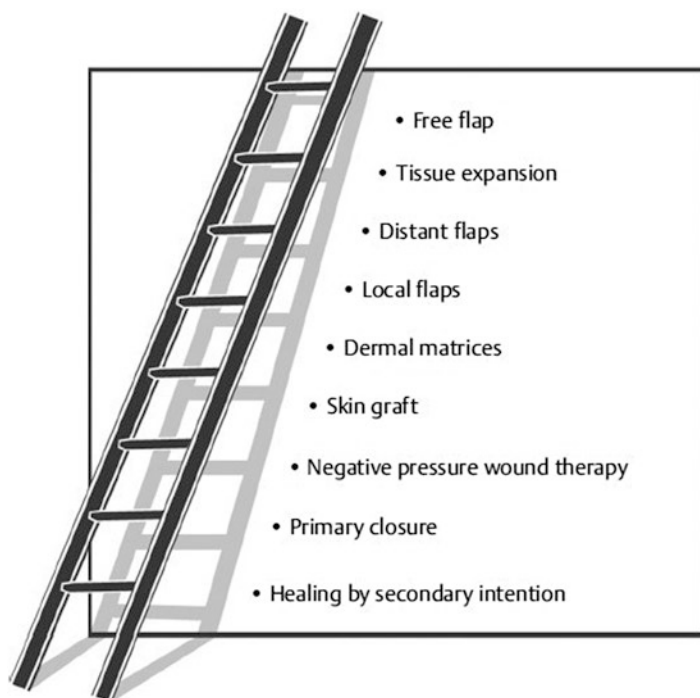
Fig. 5.6 Reconstructive ladder
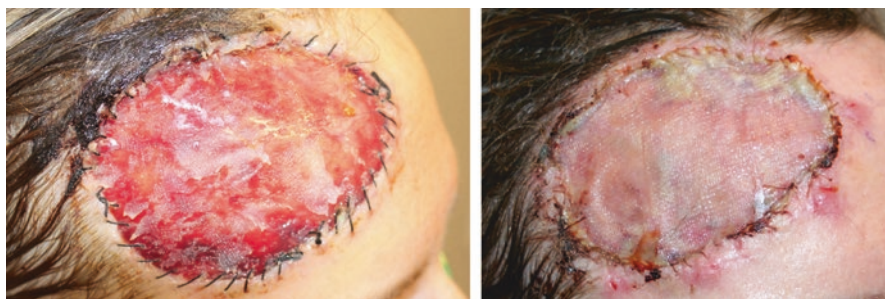
Fig. 5.7 Application of bilayered Integra over a forehead defect (left) with subsequent application of a split thickness skin graft (right)

The following is an overview of some promising technological advances beyond the mainstay of clinical use, however as this field is rapidly evolving and new advances are continuously occurring.

Advanced therapies are focused on the fundamental aspects of wound control, namely, control of infection, establishing hydrated, clean wound bed free of devital-ized tissue, and protection against future breakdown. A working understanding of wound healing biology is important to understand the effect of novel technologies. Wounds heal via three phases that interact with one another – *inflammation* where

Fig. 5.8 Application of bilayered Integra to a large anterior thigh wound (top left), removal of the silicone layer at 4 weeks (top right), and further wound healing at 6 weeks prior to skin grafting with increased vascularity and healthy granulation tissue (bottom right)

neutrophils predominate and remove debris, *proliferation* where fibroblasts form and breakdown the extracellular matrix, and *remodeling* when closed wounds develop into mature scars with decreased cell content and blood flow. Management of comorbid medical and social conditions is the base of wound care management and cellular function. Adjunctive therapies and technologies' performance is balanced against the background milieu of the patient's condition (Fig. 5.12).

Novel therapies aim to provide improved wound management (infection control, removal of devitalized tissue), recruitment (directly or indirectly) of cytokines and cell-mediated responses, and management of mature wounds.

5.7.1 Dressings

The ideal wound management should allow for oxygen permeability, clearance of exudate, protection against infection, and desiccation while providing structural and biological characteristics of the extracellular matrix (ECM) [39, 40]. Naturally occurring polymers (chitosan, alginic acid, cellulose, hyaluronic acid) are often used. Formulations of these polymers are made into dressings with nanoparticles, microparticles, films, foams, hydrogels, and nanofibers, or combinations thereof [40].

Hydrocolloids made from gelatin, pectin, or cellulose offer carbohydrate-based hydrating gels which prevent excess desiccation. These can be shaped as they adhere/mold to the specific wound. They should be used in wounds with excess or heavy exudate as excess fluid may disrupt the gel composition and lead to migration [41].
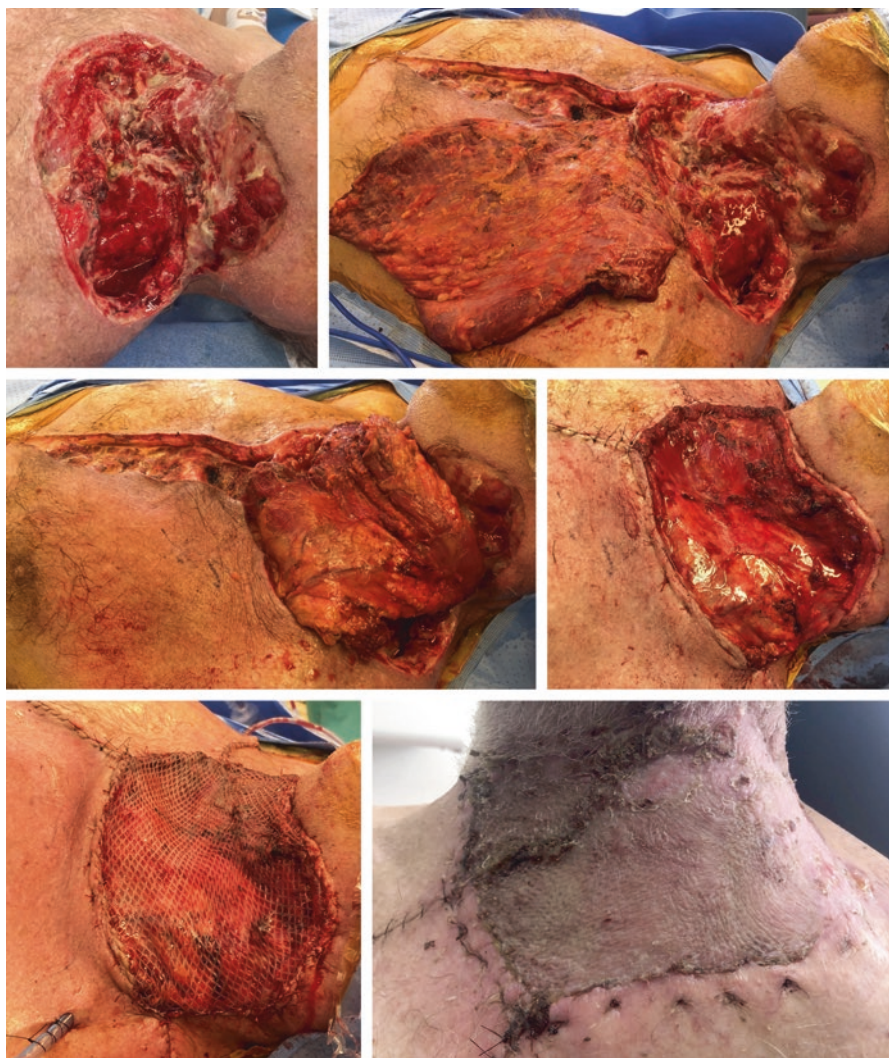
Fig. 5.9 Patient with necrotizing fasciitis of the left neck secondary to a locally advanced perforated esophageal cancer. Urgent reconstruction was required due to the exposure of vital structures and risk of vessel rupture from prolonged exposure. Patient underwent wound coverage with a left pedicled pectoralis muscle flap and split thickness skin graft

Hydrogels are water-based cross-linked hydrophilic polymers of carboxymethylcellulose and propylene glycol that "donate water" to the application sites while preventing water loss. This is thought to assist with autolytic debridement of nonviable tissue [41, 42]. Notably these may lead to maceration and may require prolonged placement for clinical effect.

Foam dressings share a spongelike architecture that allows for the absorption of exudate while being moldable to fit into irregularly shaped wounds. Importantly

Fig. 5.10 Lower extremity full-thickness wound. Key surgical principles of wound debridement, management of infection, and appropriate reconstruction, in this case with a free flap from the thigh for limb salvage

Fig. 5.11 Lower extremity wound with exposed, contaminated hardware. Key principles are removal of infected hardware with wide debridement. Temporizing measures may be taken with antibiotic beads. Once the wound is cleared of contaminated hardware, robust vascularized tissue is used for wound coverage and secondary reconstruction may proceed as needed
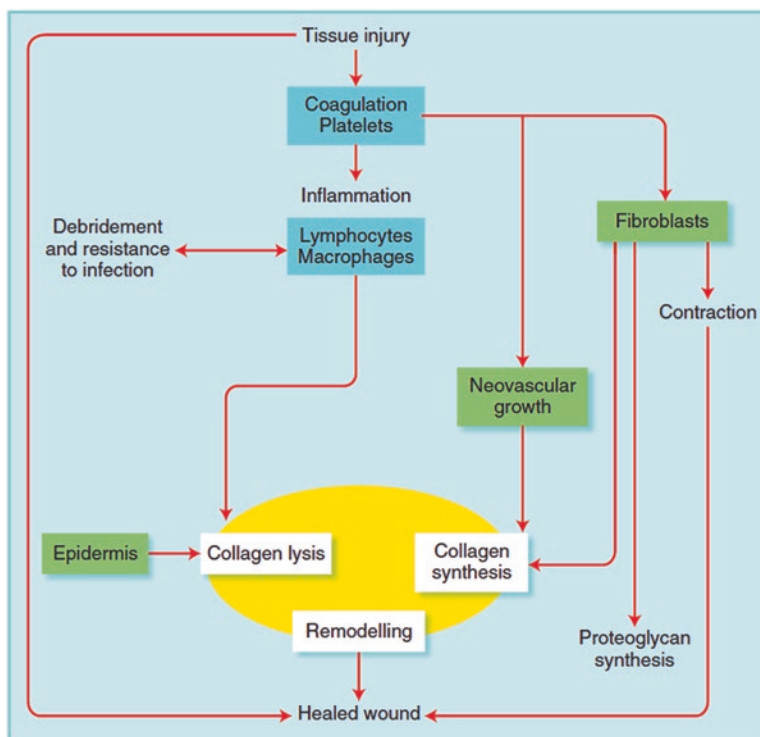
Fig. 5.12 Harding KG, Morris HL, Patel GK. Science, medicine and the future: healing chronic wounds. BMJ. 2002;324(7330):160–163. doi:https://doi.org/10.1136/bmj.324.7330.160

they must be changed when saturated as they may lead to maceration if left unchecked.

Alginate gels are viscous, hydrophilic derivatives of brown algae that are useful in high exudative wounds as they absorb water. These can be overly absorptive if not monitored.

Chitosan is a naturally occurring polymer with antimicrobial properties that promotes gas exchange and promotes wound drainage. Dressings incorporating chitosan include nano-/microparticle delivery systems and incorporation into hydrogels. Ongoing studies aim to improve the mechanical properties and antimicrobial effects of chitosan-based dressings [40, 43]. Further work is also aimed at developing 3D printed chitosan-pectin biopolymeric hydrogels [44].

Hyaluronic acid is a ubiquitous protein found in the skin and several connective tissues in the human body. It plays a vital role in wound healing and embryonic development, and is thought to maintain tissue integrity, facilitating adhesion and differentiation of cells during inflammation [45]. Several experimental hydrogels and tissue-engineered constructs are currently under study with the promise of improved wound healing, biocompatibility, and biodegradability [40, 46].

Combination polymers are another area of active study. The combination of bacterial cellulose is modifiable and allows for the introduction of drug delivery [47, 48]. Alginate and collagen backgrounds are often used for their porous properties which mimic human extracellular matrix [46, 49, 50]. Collagen-based biomaterials have been shown to elicit cytokine responses which recruit macrophages and fibroblasts [40, 51, 52].

5.7.2 Placental-Derived Membranes

The human placenta is comprised of placental membranes, amnion and chorion, which enclose the amniotic fluid and fetus. The basement membrane of the amnion is composed of type I/III collagen and fibronectin. The chorion is separated from the amnion by a thin spongy layer. Interestingly, these structures contain no blood vessels or nerves, and instead nutrients are attained by diffusion from the amniotic fluid. When used clinically, these tissues are employed either in isolation or combined, and are treated by cryopreservation, devitalized and dehydrated, or decellularized and dehydrated [53, 54]. In contrast to drugs and devices, tissue allografts do not require premarket approval, and as such, there are a growing number of placental-derived allografts in the marketplace. Although there are no randomized controlled trials, in vitro data and limited clinical studies purport improved aggregation of inflammatory and vasculogenic growth factors, and improved times to wound healing [54, 55].

Despite the significant promise of these, and a great panoply of naturally occurring and synthetic materials aimed at improving wound healing, new technologies and advances face the challenges of completing randomized controlled clinical trials to demonstrate efficacy in wound healing. Mainstay principles of controlling comorbidities (diabetes, vascular disease, nicotine avoidance, nutrition, etc.) and wound management principles (debridement, infectious control, surgical management of abscesses/necrosis) remain the keystone of wound care.

References

1. Weng, Q. Y. *et al.* Costs and Consequences Associated With Misdiagnosed Lower Extremity Cellulitis. *JAMA Dermatol* **153**, 141-146, https://doi.org/10.1001/jamadermatol.2016.3816 (2017).
2. Garcia, B. M., Cruz-Diaz, C., Agnihothri, R. & Shinkai, K. Distinguishing Cellulitis from Its Noninfectious Mimics: Approach to the Red Leg. *Infect Dis Clin North Am* **35**, 61-79, https://doi.org/10.1016/j.idc.2020.10.001 (2021).
3. Krasagakis, K. *et al.* Analysis of epidemiology, clinical features and management of erysipelas. *Int J Dermatol* **49**, 1012-1017, https://doi.org/10.1111/j.1365-4632.2010.04464.x (2010).

4. Stevens, D. L. *et al.* Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. *Clin Infect Dis* **59**, e10-52, https://doi.org/10.1093/cid/ciu444 (2014).

5. Ko, L. N. *et al.* Clinical Usefulness of Imaging and Blood Cultures in Cellulitis Evaluation. *JAMA Intern Med* **178**, 994-996, https://doi.org/10.1001/jamainternmed.2018.0625 (2018).

6. Altmayer, S., Verma, N., Dicks, E. A. & Oliveira, A. Imaging musculoskeletal soft tissue infections. *Semin Ultrasound CT MR* **41**, 85-98, https://doi.org/10.1053/j.sult.2019.09.005 (2020).

7. Collazos, J. *et al.* Cellulitis in adult patients: A large, multicenter, observational, prospective study of 606 episodes and analysis of the factors related to the response to treatment. *PLoS One* **13**, e0204036, https://doi.org/10.1371/journal.pone.0204036 (2018).

8. Cranendonk, D. R., Lavrijsen, A. P. M., Prins, J. M. & Wiersinga, W. J. Cellulitis: current insights into pathophysiology and clinical management. *Neth J Med* **75**, 366-378 (2017).

9. Israel, J. S., McCarthy, J. E., Rose, K. R. & Rao, V. K. Watch Out for Wild Animals: A Systematic Review of Upper Extremity Injuries Caused by Uncommon Species. *Plast Reconstr Surg* **140**, 1008-1022, https://doi.org/10.1097/PRS.0000000000003754 (2017).

10. Chambers, H. F. Cellulitis, by any other name. *Clin Infect Dis* **56**, 1763-1764, https://doi.org/10.1093/cid/cit126 (2013).

11. Sartelli, M. *et al.* 2018 WSES/SIS-E consensus conference: recommendations for the management of skin and soft-tissue infections. *World J Emerg Surg* **13**, 58, https://doi.org/10.1186/s13017-018-0219-9 (2018).

12. Gottlieb, M., Avila, J., Chottiner, M. & Peksa, G. D. Point-of-Care Ultrasonography for the Diagnosis of Skin and Soft Tissue Abscesses: A Systematic Review and Meta-analysis. *Ann Emerg Med* **76**, 67-77, https://doi.org/10.1016/j.annemergmed.2020.01.004 (2020).

13. Gottlieb, M. & Peksa, G. D. Comparison of the loop technique with incision and drainage for soft tissue abscesses: A systematic review and meta-analysis. *Am J Emerg Med* **36**, 128-133, https://doi.org/10.1016/j.ajem.2017.09.007 (2018).

14. Gottlieb, M., DeMott, J. M., Hallock, M. & Peksa, G. D. Systemic Antibiotics for the Treatment of Skin and Soft Tissue Abscesses: A Systematic Review and Meta-Analysis. *Ann Emerg Med* **73**, 8-16, https://doi.org/10.1016/j.annemergmed.2018.02.011 (2019).

15. Brook, I. Microbiology and management of soft tissue and muscle infections. *Int J Surg* **6**, 328-338, https://doi.org/10.1016/j.ijsu.2007.07.001 (2008).

16. Millerioux, S., Rousset, M. & Canavese, F. Pyogenic tenosynovitis of the flexor hallucis longus in a healthy 11-year-old boy: a case report and review of the literature. *Eur J Orthop Surg Traumatol* **23 Suppl 2**, S311-315, https://doi.org/10.1007/s00590-012-1147-0 (2013).

17. Greenhalgh, M. S., Iyengar, K. P., Sangani, C. & Toh, E. M. Isolated pyogenic tenosynovitis of tibialis anterior. *BMJ Case Rep* **13**, https://doi.org/10.1136/bcr-2020-236368 (2020).

18. Pang, H. N. *et al.* Factors affecting the prognosis of pyogenic flexor tenosynovitis. *J Bone Joint Surg Am* **89**, 1742-1748, https://doi.org/10.2106/JBJS.F.01356 (2007).

19. Nikkhah, D., Rodrigues, J., Osman, K. & Dejager, L. Pyogenic flexor tenosynovitis: one year's experience at a UK hand unit and a review of the current literature. *Hand Surg* **17**, 199-203, https://doi.org/10.1142/S0218810412500190 (2012).

20. Hausman, M. R. & Lisser, S. P. Hand infections. *Orthop Clin North Am* **23**, 171-185 (1992).

21. Siegel, D. B. & Gelberman, R. H. Infections of the hand. *Orthop Clin North Am* **19**, 779-789 (1988).

22. Michon, J. [Phlegmon of the tendon sheaths]. *Ann Chir* **28**, 277-280 (1974).

23. Small, L. N. & Ross, J. J. Suppurative tenosynovitis and septic bursitis. *Infect Dis Clin North Am* **19**, 991-1005, xi, https://doi.org/10.1016/j.idc.2005.08.002 (2005).

24. Kour, A. K., Looi, K. P., Phone, M. H. & Pho, R. W. Hand infections in patients with diabetes. *Clin Orthop Relat Res*, 238-244, https://doi.org/10.1097/00003086-199610000-00034 (1996).

25. Giladi, A. M., Malay, S. & Chung, K. C. A systematic review of the management of acute pyogenic flexor tenosynovitis. *J Hand Surg Eur Vol* **40**, 720-728, https://doi.org/10.1177/1753193415570248 (2015).

26. Lundberg, I. E. *et al.* 2017 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Adult and Juvenile Idiopathic Inflammatory Myopathies and Their Major Subgroups. *Arthritis Rheumatol* **69**, 2271-2282, https://doi.org/10.1002/art.40320 (2017).

27. Targoff, I. N., Miller, F. W., Medsger, T. A., Jr. & Oddis, C. V. Classification criteria for the idiopathic inflammatory myopathies. *Curr Opin Rheumatol* **9**, 527-535, https://doi.org/10.1097/00002281-199711000-00008 (1997).

28. Stevens, D. L. & Bryant, A. E. Necrotizing Soft-Tissue Infections. *N Engl J Med* **377**, 2253-2265, https://doi.org/10.1056/NEJMra1600673 (2017).

29. Bonne, S. L. & Kadri, S. S. Evaluation and Management of Necrotizing Soft Tissue Infections. *Infect Dis Clin North Am* **31**, 497-511, https://doi.org/10.1016/j.idc.2017.05.011 (2017).

30. Gozal, D., Ziser, A., Shupak, A., Ariel, A. & Melamed, Y. Necrotizing fasciitis. *Arch Surg* **121**, 233-235, https://doi.org/10.1001/archsurg.1986.01400020119015 (1986).

31. McLellan, E., Suvarna, K. & Townsend, R. Fatal necrotizing fasciitis caused by Haemophilus influenzae serotype f. *J Med Microbiol* **57**, 249-251, https://doi.org/10.1099/jmm.0.47603-0 (2008).

32. Stumvoll, M. & Fritsche, A. Necrotizing fasciitis caused by unencapsulated Haemophilus influenzae. *Clin Infect Dis* **25**, 327, https://doi.org/10.1086/516908 (1997).

33. Wong, C. H. *et al.* Necrotizing fasciitis: clinical presentation, microbiology, and determinants of mortality. *J Bone Joint Surg Am* **85**, 1454-1460 (2003).

34. Stevens, D. L. *et al.* Group A streptococcal bacteremia: the role of tumor necrosis factor in shock and organ failure. *J Infect Dis* **173**, 619-626, https://doi.org/10.1093/infdis/173.3.619 (1996).

35. Gompelman, M., van Asten, S. A. V. & Peters, E. J. G. Update on the Role of Infection and Biofilms in Wound Healing: Pathophysiology and Treatment. *Plast Reconstr Surg* **138**, 61S-70S, https://doi.org/10.1097/PRS.0000000000002679 (2016).

36. Wolcott, R. D. *et al.* Biofilm maturity studies indicate sharp debridement opens a time-dependent therapeutic window. *J Wound Care* **19**, 320-328, https://doi.org/10.12968/jowc.2010.19.8.77709 (2010).

37. Quain, A. M. & Khardori, N. M. Nutrition in Wound Care Management: A Comprehensive Overview. *Wounds* **27**, 327-335 (2015).

38. Blume, P. A., Walters, J., Payne, W., Ayala, J. & Lantis, J. Comparison of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: a multicenter randomized controlled trial. *Diabetes Care* **31**, 631-636, https://doi.org/10.2337/dc07-2196 (2008).

39. Ehterami, A. *et al.* In vitro and in vivo study of PCL/COLL wound dressing loaded with insulin-chitosan nanoparticles on cutaneous wound healing in rats model. *Int J Biol Macromol* **117**, 601-609, https://doi.org/10.1016/j.ijbiomac.2018.05.184 (2018).

40. Okur, M. E., Karantas, I. D., Senyigit, Z., Ustundag Okur, N. & Siafaka, P. I. Recent trends on wound management: New therapeutic choices based on polymeric carriers. *Asian J Pharm Sci* **15**, 661-684, https://doi.org/10.1016/j.ajps.2019.11.008 (2020).

41. Taquino, L. T. Promoting wound healing in the neonatal setting: process versus protocol. *J Perinat Neonatal Nurs* **14**, 104-118, https://doi.org/10.1097/00005237-200006000-00008 (2000).

42. Steen, E. H. *et al.* Wound Healing and Wound Care in Neonates: Current Therapies and Novel Options. *Adv Skin Wound Care* **33**, 294-300, https://doi.org/10.1097/01.ASW.0000661804.09496.8c (2020).

43. Reyes-Ortega, F. *et al.* Bioactive bilayered dressing for compromised epidermal tissue regeneration with sequential activity of complementary agents. *Acta Biomater* **23**, 103-115, https://doi.org/10.1016/j.actbio.2015.05.012 (2015).

44. Long, J. *et al.* A 3D printed chitosan-pectin hydrogel wound dressing for lidocaine hydrochloride delivery. *Mater Sci Eng C Mater Biol Appl* **104**, 109873, https://doi.org/10.1016/j.msec.2019.109873 (2019).

45. Brenes, R. A. *et al.* Hyaluronate-iodine complex: a new adjunct for the management of complex sternal wounds after a cardiac operation. *Arch Surg* **146**, 1323-1325, https://doi.org/10.1001/archsurg.2011.272 (2011).

46. Hussain, Z., Thu, H. E., Shuid, A. N., Katas, H. & Hussain, F. Recent Advances in Polymer-based Wound Dressings for the Treatment of Diabetic Foot Ulcer: An Overview of State-of-the-art. *Curr Drug Targets* **19**, 527-550, https://doi.org/10.2174/13894501186661707041325 23 (2018).

47. Portela, R., Leal, C. R., Almeida, P. L. & Sobral, R. G. Bacterial cellulose: a versatile biopolymer for wound dressing applications. *Microb Biotechnol* **12**, 586-610, https://doi.org/10.1111/1751-7915.13392 (2019).

48. Carvalho, T., Guedes, G., Sousa, F. L., Freire, C. S. R. & Santos, H. A. Latest Advances on Bacterial Cellulose-Based Materials for Wound Healing, Delivery Systems, and Tissue Engineering. *Biotechnol J* **14**, e1900059, https://doi.org/10.1002/biot.201900059 (2019).

49. Aderibigbe, B. A. & Buyana, B. Alginate in Wound Dressings. *Pharmaceutics* **10**, https://doi.org/10.3390/pharmaceutics10020042 (2018).

50. Hoseinpour Najar, M., Minaiyan, M. & Taheri, A. Preparation and in vivo evaluation of a novel gel-based wound dressing using arginine-alginate surface-modified chitosan nanofibers. *J Biomater Appl* **32**, 689-701, https://doi.org/10.1177/0885328217739562 (2018).

51. Li, X. *et al.* Functionalized silk fibroin dressing with topical bioactive insulin release for accelerated chronic wound healing. *Mater Sci Eng C Mater Biol Appl* **72**, 394-404, https://doi.org/10.1016/j.msec.2016.11.085 (2017).

52. Fleck, C. A. & Simman, R. Modern collagen wound dressings: function and purpose. *J Am Col Certif Wound Spec* **2**, 50-54, https://doi.org/10.1016/j.jcws.2010.12.003 (2010).

53. Raspovic, K. M. *et al.* Effectiveness of viable cryopreserved placental membranes for management of diabetic foot ulcers in a real world setting. *Wound Repair Regen* **26**, 213-220, https://doi.org/10.1111/wrr.12635 (2018).

54. Brantley, J. & Verla, T. Use of Placental Membranes for the Treatment of Chronic Diabetic Foot Ulcers. *ADVANCES IN WOUND CARE* **4**, 545-559 (2015).

55. Zelen, C. M., Serena, T. E., Denoziere, G. & Fetterolf, D. E. A prospective randomised comparative parallel study of amniotic membrane wound graft in the management of diabetic foot ulcers. *Int Wound J* **10**, 502-507, https://doi.org/10.1111/iwj.12097 (2013).

Chapter 6
Incidence, Complications, and Novel Treatment Strategies: Diabetic Ulcer of the Limb

Leila Yazdanpanah

Abstract Diabetes mellitus (DM) is one of the major problems in healthcare systems and a global pandemic that has increased dramatically over the past few decades (Zhang et al., J Diabetes Investig 11: 241–249, 2020; Ramachandran et al., World J Diabetes 3: 110–117, 2012). According to epidemiological studies, the number of patients with DM increased from approximately 30 million cases in 1985 to 422 million in 2014 (Whiting et al., Diabetes Res Clin Pract 94: 311–321, 2011; Shahbazian et al., Pak J Med Sci 29: 730–734, 2013). This chapter describes at-risk patients and the complications associated with diabetes in the limb including diabetic neuropathy, infection, foot deformity, and ischemia. Diabetic foot management is a multidisciplinary approach and needs a well-functioning teamwork of general physicians, and endocrinologists, as well as specialists in infectious disease, vascular surgery, orthopedics, intervention, orthotics, and prosthetics and educated nurses. This chapter discusses the basic treatment strategies as well as highlights novel and emerging treatment approaches that may improve clinical outcome in the future.

Keywords Diabetes · Infection · Diabetic neuropathy · Diabetic foot infection · Inflammation · Osteomyelitis · Charcot foot · Diabetic ischemia · Treatment · Novel strategies

6.1 At-Risk Patients

Diabetes mellitus (DM) is one of the major problems in healthcare systems and a global pandemic that has increased dramatically over the past few decades [1, 2]. According to epidemiological studies, the number of patients with DM increased from approximately 30 million cases in 1985 to 422 million in 2014 [3, 4]. These figures are alarming because an increase in diabetes prevalence will increase the

L. Yazdanpanah (✉)
Health Research Institute, Diabetes Research Center, Ahvaz Jundishpur University of Medical Sciences, Ahvaz, Iran
e-mail: yazdanpanah.l@ajums.ac.ir

number of acute and chronic complications in the general population, bringing about enormous effects on quality of life and economic burdens [5].

As far as complications associated with diabetes are concerned, diabetic foot management remains a major challenge for healthcare professionals. The diabetic foot is still the most frequent cause of hospitalization of patients with diabetes, and diabetes accounts for 70% (more than half) of nontraumatic amputations in the world [6–8]. A few years ago, a lower limb was amputated worldwide due to diabetes every 30 seconds, but unfortunately today this figure has become 20 seconds as a result of the rapid prevalence of diabetes [6, 9, 10]. A diabetic foot ulcer (DFU) imposes significant financial burdens on public and private paymasters, ranging from $9 to $13 billion, in addition to the costs associated with diabetes itself. Healing of a single DFU is estimated to cost $17,500 USD, and if it leads to amputation, the cost will go as high as $30,000–33,500 USD. Additionally, there are indirect costs including disability, rehabilitation, home care, etc. Therefore, nearly 7–20% of diabetes-related expenditure in North America and Europe is due to DFU costs. The global DFU market expects a positive 6.6% compound annual growth rate between 2016 and 2024. At this pace, the market's estimation may reach $4.9 billion by the end of 2024 [10, 11].

The global DFU prevalence is about 1.3–12% in different studies with an average of 6.3% [10, 12]. About 15–25% of patients with diabetes may develop a foot ulcer in their lifetime. The annual risk of developing a diabetic foot ulcer in patients with diabetes is appraised to be about 2%, but this risk in patients with a previous history of foot ulceration is expected to increase to 17–60% over the subsequent 3-year period [10, 13, 14]. Recent studies have shown multiple risk factors related to DFU development [13–15]. These risk factors are (i) duration of diabetes >10 years, (ii) gender (male), (iii) high body mass index (BMI), (iv) age, and (v) comorbidities such as diabetic peripheral neuropathy, peripheral arterial disease (PAD), foot deformity, diabetic retinopathy, metabolic control (e.g., hemoglobin, HbA1C), infections, unsuitable footwear, and reduced self-care behaviors [4, 14–16].

While the literature has recognized a number of diabetes-related risk factors that lead to lower-extremity ulceration and amputation, most DFUs have been caused by neuropathy, ischemia, or foot deformities. Pure ischemic ulcers probably represent only 10% of DFUs, while 90% are caused by neuropathy, alone or with ischemia. Neuro-ischemic ulcers are the most common type of ulcers seen in diabetic foot clinics today [6, 17, 18].

Today, various investigations have shown that elevated plantar pressures are associated with foot ulceration. Moreover, it has been proven that foot deformities and gait instability increase plantar pressure, which can result in foot ulceration [19, 20] (Fig. 6.1). Unfortunately, frequently patients with diabetes tend to deny their disease and fail to take part in the self-management of their disease. However, several studies have shown that appropriate management of a DFU can significantly reduce, delay, or prevent complications such as infection, gangrene, amputation, and even death [17, 20, 21].

As diabetes is a multi-organ systemic disease, all comorbidities that influence wound healing must be managed by a multidisciplinary team for ideal outcomes for
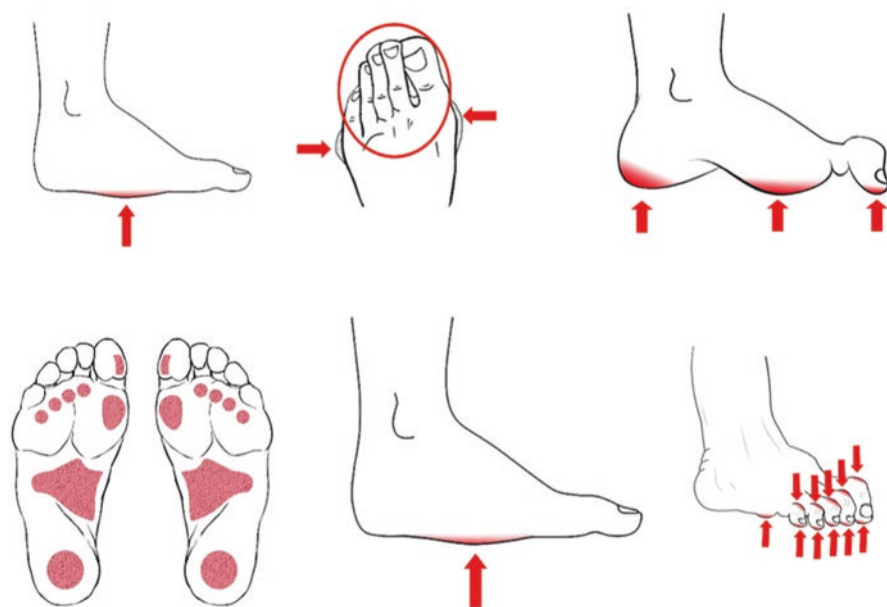
Fig. 6.1 Areas of the foot at highest risk for ulceration (from IWGDF guideline 2019 [21])

the DFU. Currently, several studies have shown that a multidisciplinary team can decrease amputation rates, lower costs, and lead to better quality of life for patients with a DFU. A preventive care team, defined as a multidisciplinary team, can decrease the risks associated with DFU and amputation by 50–85% [21–26].

6.2 Diabetic Neuropathy

Neuropathy is the most common complication of DM, affecting up to 50% of patients. Its prevalence is different in terms of the two types of diabetes. Previous studies have reported prevalence rates for polyneuropathy to vary from 8% to 54% in type 1 diabetic patients and from 13% to 46% in type 2 patients with diabetes [27, 28]. It may present at the beginning of a diabetes diagnosis in about 10% of patients, and it may even present in prediabetes patients. Peripheral neuropathy is the most common risk factor for DFUs, promoting more than 80% of these ulcers [29–31]. The most common type of neuropathy in patients with diabetes is sensory-motor distal symmetric neuropathy. Sensory neuropathy illustrates a stocking-and-glove distribution in the distal limbs. Sensory symptoms may be positive or negative, and focal or diffuse. Negative sensory symptoms manifest loss of sensation due to axon/neuron loss, which consists of feelings of numbness and loss of balance. Positive symptoms reflect abnormal excitability of the nervous system and may be described as tingling, burning, pricking pain, tightness, or hypersensitivity to touch. Absent or

decreased ankle reflexes happen early in the disease, though more extensive loss of reflexes and motor weakness are late findings [27, 31]. Besides, motor neuropathy can lead to foot deformity (see Sect. 6.4).

Diabetic neuropathy is known to affect injury to both large-diameter, myelinated nerve fibers and small-diameter, unmyelinated nerve fibers. Small-diameter nerve fibers correspond to 70–90% of all peripheral nerve fibers and are supposed to be the earliest fibers to be impaired in diabetes, causing temperature, pain, and pressure sensation disorder. Large fiber involvement is seen in vibration perception disorder [30, 32, 33]. Thus, neurologic examination should be taken into account as the first and the most important screening tool in patients at risk of developing a foot ulcer because diabetic neuropathy can lead to DFU, lower limb infections, and amputation [31]. The most important issue in this regard is identifying at-risk patients by screening loss of protective sensation (LOPS). LOPS assessment is suggested with one of the following techniques:

1. *Pressure perception*: use of the 10 gram monofilament.

The Nylon 10 g Semmes-Weinstein monofilament is applied perpendicular on the three different sites as shown in Fig. 6.2. To this aim, the filament is pressed onto the skin, and the patient is asked whether they feel the pressure applied ("yes"/"no"). The total interval of the test should be approximately 2 seconds. Areas of ulcer, calluses, necrotic tissues, and scars are avoided during the test. Protective sensation is present at each site if the patient accurately answers on two out of three applications, and it is absent when two out of the three answers are inaccurate [21].

2. *Vibration perception*: 128 Hz tuning fork

In this test, the tuning fork is applied onto a bony part on the dorsal side of the distal phalanx of the first toe (or another toe if the hallux is absent). The tuning fork is applied perpendicularly, with persistent pressure (Fig. 6.3). It is necessary to make sure that the patient cannot see whether or where the examiner applies the tuning fork. The test is positive if the patient accurately answers at least two out of three applications and negative if two out of three answers are inaccurate [21].
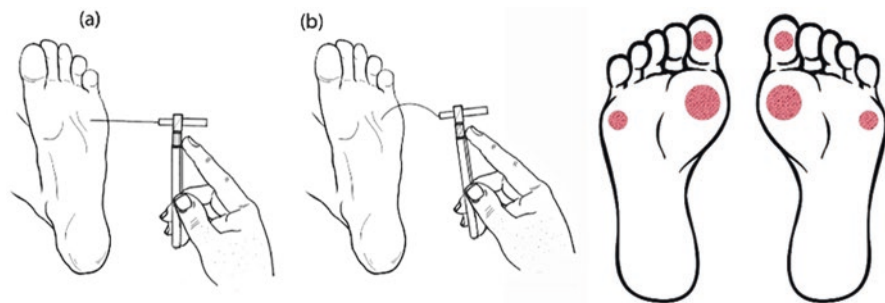
Fig. 6.2 10g monofilament test and its examination sites

Fig. 6.3 128 Hz tuning fork test
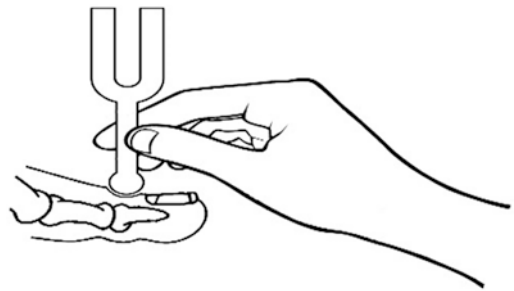
Table 6.1 The IWGDF 2019 risk stratification system [21]

Category	Ulcer risk	Characteristics	Frequency
0	Very low	No LOPS and no PAD	Once a year
1	Low	LOPS or PAD	Once every 6–12 months
2	Moderate	LOPS + PAD, or LOPS + foot deformity or PAD + foot deformity	Once every 3–6 months
3	High	LOPS or PAD, and one or more of the following: – History of a foot ulcer – A lower-extremity amputation (minor or major) – End-stage renal disease	Once every 1–3 months

All patients with diabetes (type 2 at the time of diagnosis and type 1 5 years after diagnosis) have to be examined through neurologic screening every year using a 10 g monofilament sensation, vibration perception, and pain and temperature sensation test. After examination, we can stratify patients in different risk groups to manage their follow-ups according to Table 6.1.

6.3 Diabetic Foot Infection

Diabetic foot infections (DFIs) are associated with considerable morbidities, requiring daily wound care, antimicrobial therapy, repeated healthcare provider visits, and surgical procedures, all of which imposing high healthcare costs [34]. DFIs continue to be the most common cause of hospitalization in patients with diabetes and the most common reason for lower-extremity amputation [35, 36]. Approximately more than half of DFU patients (nearly 60%) develop infection [37, 38]. In more than two-thirds of the cases, infection is the principal reason for major lower limb amputation in patients with a DFU. The prevalence of DFI has been reported to be about 25–60%. It is reported that patients who have a DFI are 155 times more likely to experience amputation compared with those who do not have infection. Nearly

20% of moderate and severe DFIs result in amputation [38, 39]. Infections may complicate DFUs not only those of the neuropathic type but also ischemic ulcers. Outcomes in patients with an infected diabetic foot ulcer are poor. For example, in one large prospective study at the end of the first year, ulcers healed in only 46% of patients, while 15% expired and 17% required a lower-extremity amputation. Hence, global research on diabetic foot ulcers shows that DFI is the most frequent topic in this respect [36, 40, and 41].

6.3.1   Identification of a DFI

Even though a wide variety of bacteria may colonize foot ulcers, infection is taken into consideration just as an inflammatory reaction occurs, because of the interface between bacteria and host tissues. Colonization is usually restricted to the skin surface, while infection is characterized by the involvement of subcutaneous or the deeper tissue layers. DFI management requires carefully diagnosing the condition, obtaining proper specimens for culture, thoughtfully choosing antimicrobial therapy, rapidly determining when surgical interventions are needed, and providing wound and overall patient care. A systematic, evidence-based approach to managing DFIs improves outcomes and avoids complications such as lower-extremity amputation. Multidisciplinary teams would better include an infectious diseases or medical microbiology specialist. Such teams should try to ensure optimal local wound care (e.g., cleansing and debridement), pressure offloading, vascular assessment, and metabolic (predominantly glycemic) control. Numerous guidelines are available to assist clinicians in managing DFIs [42].

Infection is best defined as an invasion and multiplication of microorganisms in host tissues that induces a host inflammatory response, usually followed by tissue destruction [21, 43]. In patients with a DFU, deep tissues are exposed to bacterial colonization, and immediately the protective layer of the skin is ruptured. Infection in a diabetic foot cannot be described merely in terms of wound culture results. Therefore, the presence of inflammatory signs in any type of foot tissue in a patient with diabetes is considered a DFI. Nevertheless, in patients with diabetes, some inflammation symptoms or signs may be masked because of diabetic neuropathy, immunity dysfunction, or presence of PAD. Limb ischemia and diabetic neuropathy increase the risk of an ulcer becoming infected and more complicated [35, 44–47]. Some of the predisposing factors of foot infection in these patients include deep, recurrent, or long-standing ulcers, chronic renal failure, and persistent hyperglycemia [46, 48]. Because of the nature of foot anatomy which includes separate but intercommunicating compartments, infection may lead to compartmental pressure, ischemic tissue necrosis, and progressive infection [49, 50].

According to one classification, DFIs are classified into non-limb-threatening, limb-threatening, and life-threatening infections. Non-limb-threatening infections are superficial without ischemia and osteomyelitis. In this type of infection, the cellulitis around the ulcer is ≤2 cm, and the patient is clinically stable so they can be

supervised in outpatient service. Limb-threatening infections in patients with diabetes may present along with fever, limb edema, lymphangitis, hyperglycemia, leukocytosis, and ischemia. Cellulitis in these patients is ≥2 cm, and the probe test may be positive, and osteomyelitis may be present. Consequently, if there is any gangrene, abscess, osteomyelitis, or necrotizing fasciitis, the patient has to be admitted for inpatient services [43, 51, 52]. If not treated properly, DFIs have a tendency to progress, causing osteomyelitis and limb- or life-threatening infections. For their convenience, healthcare providers are recommended to use the SINBAD classification [Table 6.2]. To categorize DF infections, IWGDF suggests this classification [Table 6.3].

6.3.2 Osteomyelitis

Osteomyelitis (OM) is a common consequence of a DFU infection. It presents in 10–15% of moderate and in 50% of severe infections, and may underlay any DFU, particularly those that are chronic for many weeks, causing an erythematous, swollen ("sausage") toe. OM is principally the outcome of a soft tissue infection that extends to the bone, involving first the cortex and afterward the marrow [38, 40].

Osteomyelitis can involve any bone, but largely involvement is in the forefoot (90%), followed by the midfoot (5%) and the hindfoot (5%). Prognosis in forefoot OM is better than midfoot and hindfoot osteomyelitis. Above-the-ankle amputation risk is considerably greater for hindfoot (50%) than midfoot (18.5%) and forefoot (0.33%) [53, 54].

Table 6.2 SINBAD classification of DFU [21]

Category	Definition	Score
Site	Forefoot	0
	Midfoot and hindfoot	1
Ischemia	Pedal blood flow intact: At least one palpable pulse	0
	Clinical evidence of reduced pedal flow	1
Neuropathy	Protective sensation intact	0
	Protective sensation lost	1
Bacterial infection	None	0
	Present	1
Area	Ulcer <1 cm^2	0
	Ulcer ≥1 cm^2	1
Depth	Ulcer confined to the skin and subcutaneous tissue	0
	Ulcer reaching the muscle, tendon, or deeper	1
Total possible score		6

Table 6.3 The classification system to define the presence and severity of DFIs [21]

Clinical classification of infection, with definitions Uninfected	IWGDF classification
No systemic or local symptoms or signs of infection	I (uninfected)
Infected	

At least two of these items are present:
  • Local swelling or induration
  • Erythema >0.5 cm* around the wound
  • Local tenderness or pain
  • Local increased warmth
  • Purulent discharge
And no other cause(s) of an inflammatory response of the skin (e.g., trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, or venous stasis)	

Infection with no systemic manifestations (see below) involving:
  • Only the skin or subcutaneous tissue (not any deeper tissues)
  • Any erythema present does not extend >2 cm** around the wound
	2 (mild infection)

Infection with no systemic symptoms, and involving:
  • Erythema extending ≥2 cm* from the wound margin
  • Tissue deeper than the skin and subcutaneous tissues (e.g., tendon, muscle, joint, bone)
	3 (moderate infection)

Any foot infection with associated systemic manifestations (of the systemic inflammatory response syndrome [SIRS]), as manifested by ≥2 of the following:
  • Temperature > 38 $^\circ C$ or <36 $^\circ C$
  • Heart rate > 90 beats/minute
  • Respiratory rate > 20 breaths/minute or $PaCO_2$ <4.3 kPa (32 mmHg)
  • White blood cell count >12,000/mm^3, or > 10% immature (band) forms
	4 (severe infection)

Infection involving the bone (osteomyelitis)	Add "(O)" after 3 or 4***

Note: * Infection refers to any part of the foot not just of a wound or an ulcer, ** in any direction, from the rim of the wound. The presence of clinically significant foot ischemia makes both diagnosis and treatment of infection considerably more difficult. *** If osteomyelitis is demonstrated in the absence of ≥2 signs/symptoms of local or systemic inflammation, classify the foot as either grade 3(O) (if <2 SIRS catena) or grade 4(O) if ≥2 SIRS catena)

OM diagnosis is difficult because there is no uniquely identified description all over the world [55, 56]. At least two signs of inflammation have to be present; however, diabetic foot OM can appear without any local sign of inflammation. Two certain clinical signs are used to predict osteomyelitis. The first is the foot ulcer size (width and depth). Ulcers larger than 2 cm have a sensitivity of 56% and a specificity of 92%. Deep ulcers (> 3 mm) are more correlated with a fundamental OM than superficial ulcers (82% vs. 33%). The probe-to-bone (PTB) test is the second way that is the most helpful test to diagnose OM. However, its reliability depends on the clinician's skill and the ulcer's location and etiology. The test requires just a sterile blunt metal probe mildly inserted into the wound, with a positive test expressed by sensation of a hard structure. PTB has demonstrated a sensitivity of 66–87%, a specificity of 85–91%, a positive predictive value of 57–89%, and a negative

predictive value of about 98% in different studies [38, 40, 57, 58]. If the PTB test is positive in a high-risk patient and negative in a low-risk patient, it is reliable to diagnose DFIs. Therefore, in the infected ulcer, a positive PTB test is greatly suggestive of OM, but a negative test does not exclude it. As an alternative, in an ulcer without infection, a positive test may not be specific to OM. However, a negative PBT test should exclude a bone infection [37, 59, 60]. (Table 6.4)

6.3.3 Serum Inflammatory Indicators

Serum inflammatory indicators such as white blood cells (WBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and procalcitonin (PCT) are usually higher in OM than soft tissue infections. WBC count has been reported to have a slight correlation with infection severity in many studies. About half of the cases of DFI have normal WBC [52, 61]. Another indicator, ESR, in most studies, has been higher in patients with DFIs in comparison with diabetic foot ulcers without infection. Nevertheless, ESR values may be affected by a number of conditions such as anemia and azotemia, and they may not rise in the acute phase of infection. In case of the presence of OM, and when ESR > 60 mm/h and/or CRP > 3.2 mg/dL, WBC and procalcitonin may be within normal limits. However, an ESR ≥ 70 mm/h is more common in bone infections in comparison with soft tissue infections. Procalcitonin is a peptide precursor of the calcitonin hormone which is often undetectable or has low concentrations in healthy populations. Several tissues (kidney, adipose tissue, lung, and liver) produce PCT in the presence of infection, and the blood concentration can rise. PCT is considered positive if ≥0.5 ng/ml [38, 62–65].

WBC, CRP, and PCT values return to their normal range approximately 3 weeks after treatment in both soft tissue and bone infections. However, ESR frequently stays high and only in the presence of osteomyelitis. CRP levels are predisposed to

Table 6.4 IWGDF/IDSA system

Clinical manifestations	Infection severity	PEDIS grade
Wound lacking purulence or any manifestations of inflammation	Uninfected	1
Presence of ≥2 manifestations of inflammation (purulence, or erythema, tenderness, warmth, or induration), but any cellulitis/erythema extends ≤2 cm around the ulcer, and infection is limited to the skin or superficial subcutaneous tissues; no other local complications or systemic illness	Mild	2
Infection (as above) in a patient who is systemically well and metabolically stable but which has ≥1 of the following characteristics: cellulitis extending >2 cm, lymphangitic streaking, spread beneath the superficial fascia, deep-tissue abscess, gangrene, and involvement of muscle, tendon, joint, or bone	Moderate	3
Infection in a patient with systemic toxicity or metabolic instability (e.g., fever, chills, tachycardia, hypotension, confusion, vomiting, leukocytosis, acidosis, severe hyperglycemia, or azotemia)	Severe	4

increase more rapidly with infection and decrease faster in resolving infection. Serum levels of CRP are constantly higher in DFIs than those in diabetic foot ulcers without infection, and its levels increase notably with the severity of infection. Among the inflammatory markers, CRP has shown higher diagnostic accuracy than WBC and ESR [38, 63, 66, 67].

6.3.4   Imaging in the Diagnosis of DFI and Osteomyelitis

All patients suspected of having osteomyelitis should undergo plain X-rays of the foot. Plain X-rays are usually available, which are comparatively inexpensive and cause fewer side effects [68, 69]. Characteristic findings of bone infection in plain X-rays, as shown in Table 6.5, are greatly indicative for osteomyelitis, but X-rays are often negative in the first few weeks of infection because clear signs associated with osteomyelitis are mostly not apparent until 30–50% of the bone is involved, which typically occurs after 2–3 weeks. In this period, magnetic resonance imaging (MRI) with gadolinium has very great sensitivity (90%) and specificity (85%) in the diagnosis of OM. Gadolinium uptake is superior when discriminating between soft tissues and bone when compared with CT and scintigraphic methods. MRI is a test available for most patients and provides information about both soft tissue and bone infections in the foot. The main changes within the bone marrow which contribute to the diagnosis of osteomyelitis are low signal intensity on T1-weighted sequences and high signal intensity on T2-weighted sequences [37, 38].

Scintigraphic examinations are more sensitive than X-ray during the primary stage of bone infection and the follow-up. Nevertheless, their limitation is the low specificity in the contrast between soft tissues and bone infection. Labeled leukocyte imaging is more valuable than a bone scan as far as diagnosis, bone assessment,

Table 6.5 Features characteristic of diabetic foot osteomyelitis on plain X-rays [21]

New or evolving radiographic features* on serial radiographs**, including: • Loss of bone cortex, with bony erosion or demineralization   • Focal loss of trabecular pattern or marrow radiolucency (demineralization)   • Periosteal reaction or elevation   • Bone sclerosis, with or without erosion
Abnormal soft tissue density in the subcutaneous fat, or gas density, extending from the skin toward the underlying bone, suggesting a deep ulcer or sinus tract
Presence of sequestrum: Devitalized bone with radiodense appearance separated from normal bone
Presence of involucrum*: Layer of new bone growth outside previously existing bone resulting and originating from stripping off the periosteum
Presence of cloacae*: Opening in the involucrum or cortex through which sequestrum or granulation tissue may discharge

Note: *Some features (e.g., sequestrum, involucrum, and cloacae) are seen less frequently in diabetic foot osteomyelitis than in younger patients with osteomyelitis of larger bones. **Usually spaced several weeks apart

and follow-up are concerned. It has been demonstrated that combined 99mTc white blood cell-labeled single-photon emission computed tomography and computed tomography (99mTc WBC-labeled SPECT/CT) imaging give good spatial resolution with the three-dimensional CT-scan images and WBC uptake intensity producing more evidence with respect to the site and expansion of the infection. The role of 99mTc WBC-labeled SPECT/CT has been positively appraised in distinguishing the entire resolution of infection in the course of following up patients treated by antibiotics. The positron emission tomography-computed tomography (PET/CT) with fluorine-18-fluorodeoxyglucose (18F-FDG) is an outstanding hybrid imaging that can be used in OM diagnosis and in differentiating bone from soft tissue infections. For the diagnosis of OM, it is recommended to use a combination of various diagnostic tests such as PTB, serum inflammatory markers, X-ray, MRI, and radionuclide scanning. The first type of imaging should always be X-ray evaluation, but when more detailed imaging is needed, MRI is the first alternative. A white blood cell-labeled radionuclide scan, SPECT/CT, and 18F-FDG PET/CT are used in the cases where MRI is contraindicated [37, 38, 70, 71].

It should be noted that if a diabetic patient is suspected of osteomyelitis, and plain X-rays and laboratory results with clinical judgment are strongly suggestive of osteomyelitis, no additional imaging is recommended [72–75].

6.3.5 Microbiology in DFIs

It may be difficult to identify when DFIs have been successfully treated. Some inflammatory markers and plain X-rays can be helpful in the diagnosis of DFIs, but it should be noted that DFIs are not cured until at least 1 year after their healing and when there is no evidence of infection recurrence. However, if these tests show improvement and not resolution, then this should be considered solely as remission because infection recurrence at a similar location is not uncommon [76, 77].

In a diabetic patient with suspected osteomyelitis of the foot, if it is possible, gathering a sample of bone (percutaneously or surgically) for culture is helpful. The gold standard for the diagnosis of osteomyelitis is bone biopsy based on which histological findings can be obtained. This approach is a standard method used to establish the causative pathogen. It does not matter to receive antibiotic therapy before a bone culture, because in many studies this does not seem to diminish the positive cultures ratio. Obtaining a bone biopsy from all cases is ideal; however, this is of course not always possible as the procedure requires experience, time, and added cost. Nevertheless, it is essential to perform a bone biopsy when it is challenging to predict the causative pathogen. A biopsy may not be required if a deep-tissue sample collected aseptically from a soft tissue infection develops only a definite virulent pathogen, especially *Staphylococcus aureus* [38, 78, 79].

Wound swabs provide less clinically useful information on pathogen growth than a wound tissue sample (obtained by curettage or biopsy after cleansing and debridement) in DFIs. Interestingly, the identification of corresponding bacteria isolated

from both a bone biopsy and swab culture is approximately 38%. However, molecular microbiology techniques are not recommended as a first-line measure for pathogen identification. In low-income countries without structured access to culture, a Gram-stain smear of material from a DFI is a low-cost method used to identify the class of the probable causative pathogens, therefore aiding when choosing a suitable empiric therapy [37, 80, 81].

Wound recovery outcome significantly depends on qualitative and quantitative characteristics of the wound microbiology which are critical contributing factors. Wound culture results of a DFI are often polymicrobial (involving both aerobes and anaerobes). Among these microbes, isolated virulent pathogens (e.g., *Staphylococcus aureus* or beta-hemolytic streptococci) have to be treated, whereas some less virulent germs (e.g., corynebacteria or coagulase-negative staphylococci) are frequently colonized and may not necessarily need to be treated with antibiotics [21, 82]. The most commonly detected bacteria in diabetic foot OM are *Staphylococcus aureus* (up to 50% of cases), *Staphylococcus epidermidis* (about 25%), streptococci (about 30%), and *Enterobacteriaceae* (up to 40%), all of which acutely infect skin ruptures. Among the common Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus*, and *Pseudomonas aeruginosa* are the most common microorganisms. Anaerobes are often present in mixed infections particularly in cases of deep-tissue infection with aerobes, and their rate is usually low. These mixed infections can result in microbial synergy and additional increased severity of infection. It is usually supposed that acute infections that have not been previously treated with antibiotics are monomicrobial whereas chronic infections previously treated with antibiotics are polymicrobial. Hospitalization, surgical procedures, and long antibiotic therapy have contributed to the development of multiresistant organisms or methicillin-resistant *Staphylococcus aureus* (MRSA). Diabetes itself is one of the most predisposing factors of MRSA infections [37, 82–84].

6.3.6 Antibiotic Therapy and Treatment in DFIs

Antibiotics used for treating a DFI should be selected based on the following criteria: the probable or confirmed causative pathogen(s) and their antibiotic sensitivities; issued evidence of effectiveness of the agent for DFIs; the severity of the infection; risk of side effects including damage to the flora; possibility of drug interactions; agent availability; and economic costs. In all cases, treatment of an infected diabetic foot wound should be focused on a narrow spectrum of pathogen cover, preferably ordered by culture results. Agents to consider include cephalosporins, clindamycin, co-amoxiclav, quinolones, piperacillin/tazobactam, carbapenems, penicillin, metronidazole (in combination with other antibiotics), linezolid, and vancomycin [21, 40].

Any patient with a severe DFI must be treated primarily using a parenteral method. If the patient is clinically recovering and there is no contraindication to the

use of suitable and available oral agents, the treatment can be switched to the oral route. Patients with a mild and often moderate DFI can be treated orally initially. It is not recommended to use any topical antimicrobial agent only when treating a mild DFI [21, 85].

When treating a diabetic foot infection, it is recommended to maintain antibiotic therapy for 1–2 weeks. In some conditions such as widespread infection, slow resolving of infection, and severe peripheral artery disease, treatment may need to continue for up to 3–4 weeks. If the infection resolving lasts more than 4 weeks, it is suggested to re-evaluate the patient to decide on a treatment change or undertaking more diagnostic tests. Antibiotic therapy in diabetic foot osteomyelitis should be no longer than 6 weeks. Of course, the optimal duration of antibiotic therapy has not been absolutely determined. According to recommendation by the Infectious Diseases Society of America (IDSA), 4–6 weeks is sufficient when the infected bone is not totally removed by surgery, whereas in case of antibiotic therapy alone, at least 3 months may be needed. The up-to-date report from the International working Group on the Diabetic Foot (IWGDF) suggests 6 weeks of antibiotic therapy if the infected bone is not removed by surgery and no more than a week if the infected bone is resected. Both the 2012 IDSA and the 2016 IWGDF guidelines recommend that at least 4 weeks of antibiotic therapy is needed if infected or necrotic bone is present. The aim is to restrict the antibiotic therapy period to reduce undesirable outcomes. In addition, the following questions should be taken into account: Were all probable pathogens covered by taking antibiotics? Was the antibiotic taken according to prescription? Is the perfusion of peripheral arteries and intestinal absorption of antibiotic agent sufficient or impaired? Could there be any indication for surgery (e.g., an abscess, a foreign body, osteomyelitis, etc.) [21, 40, 86–89]? In most patients with DFI, empiric antibiotic therapy is considered to cover probable pathogens. The most common pathogens in DFIs are aerobic Gram-positive cocci, specifically *S. aureus*, and less significant ones are streptococci and coagulase-negative staphylococci. For patients who live in a mild to moderate climate area, empiric antibiotic therapy has to cover aerobic Gram-positive pathogens (beta-hemolytic streptococci and *Staphylococcus aureus*) in a mild diabetic foot infection without any recent usage of antibiotic therapy. Meanwhile, for patients living in a hot or humid climate, receiving antibiotic therapy in a few weeks, or having a severe ischemic limb or a moderate or severe infection, it is recommended to use empiric antibiotic regimen that covers Gram-positive pathogens, the usual isolated Gram-negative pathogens, and the probable obligate anaerobes in moderate to severe diabetic foot infections. *Pseudomonas aeruginosa* must be treated with an appropriate antibiotic if it has been identified in a previous culture or if the foot is in repeated contact with water. Meanwhile, obligate anaerobes must be considered in abscesses and ischemic limbs. These pathogens can be treated with an imidazole (metronidazole), or β-lactam with beta-lactamase inhibitor as empiric therapy (see Table 6.5). Subsequently, according to clinical response and culture result, empiric antibiotic therapy can be changed to a suitable treatment [91–94].

A 20–30% prevalence of MRSA in some countries has given currency to the use of non-β-lactam antimicrobial agents such as rifampicin, fusidic acid, trimethoprim,

and sulfamethoxazole, despite their many side effects (e.g., affecting cellular insulin sensitivity which increases the risk of hypoglycemia, peripheral neuropathy, and serotonin syndrome by co-prescription with SSRIs, etc.). Linezolid can be prescribed with caution for outpatient management. The novel cephalosporin agent ceftaroline fosamil has been demonstrated to be effective in intravenous treatment of Gram-positive infections, including MRSA, and has been valuable in the treatment of diabetic foot infections. Quinolone antibiotics, including ciprofloxacin, represent a suitable oral choice for Gram-negative cover and, in combination (e.g., rifampicin) for treatment of *S. aureus*, show good tissue penetration within the skin and soft tissue including bone. Levofloxacin has parallel bone penetration with ciprofloxacin and can be used in some Gram-positive bone infections. Fluoroquinolone antibiotics should be used carefully in patients with risk factors for QT interval prolongation (e.g., co-prescription of some antidepressants), and ECGs are prescribed in these conditions before and after treatment [37, 40].

Chronic kidney disease (CKD) is a usual comorbidity in people with DFU that increases foot ulceration risk. Nephrotoxicity of some agents such as gentamicin needs close monitoring. Several side effects such as hyperkalemia can occur more frequently in CKD patients. Therefore, renal function tests have to be considered in empiric antibiotic therapy. Sometimes it is challenging to distinguish whether a diabetic foot ulcer is infected, in particular in peripheral neuropathy or peripheral artery disease. Hence, some secondary signs or symptoms (e.g., ulcer undermining, high odor, or quantity of exudate) may be helpful. It is recommended not to treat clinically uninfected foot ulcers with systemic or even local antibiotic therapy since about half of all DFUs are uninfected at the beginning. Unnecessary antibiotic therapy can be destructive for the patient, the healthcare system, and the population overall [21, 40, 94]. (Table 6.6)

Many patients with DFIs do not need to be hospitalized, but according to the following reasons, some may need to be hospitalized:

- Need to obtain diagnostic procedures (vascular assessment, etc.)
- Complex foot infection requiring intensive assessment (such as urgent surgery, widespread gangrene, compartment syndrome, or deep abscess)
- Parenteral antibiotic therapy
- Fluid resuscitation
- Multidisciplinary approach and consultation
- Metabolic control
- Presence of comorbidities such as renal failure, immunosuppression situation, etc.
- Psychological, social, or physical disabilities

Fortunately, most of mild and moderate DFIs can be treated in the outpatient setting, and involvement of the bone does not inevitably require hospitalization except for significant associated soft tissue infection or for surgical treatment [21, 88, 95–97]. Indications for hospitalization in DFIs are shown in Table 6.7 [21].

Table 6.6 How to select an empiric antibiotic regimen for diabetic foot infections [21]

Infection severity	Additional factors	Usual pathogen(s)*	Potential empirical regimens**
Mild	No complicating features	GPC	S-S pen; first-gen ceph
	β-lactam allergy or intolerance	GPC	Clindamycin; FQ; T/S; macrolide; doxy
	Recent antibiotic exposure	GPC + GNR	β-L-ase-l T/S; FQ
	High risk for MRSA	MRSA	Linezolid; T/S; doxy; macrolide
Moderate or severe*	No complicating features	GPC±GNR	β-L-ase 1; second-/third-gen ceph
	Recent antibiotics	GPC±GNR	β-L-ase 2; third-gen ceph; group l carbapenem (depends on prior therapy; seek advice)
	Macerated ulcer or warm climate	GNR, including *Pseudomonas*	β-L-ase 2; S-S pen + ceftazidime; S-S pen + cipro; group 2 carbapenem
	Ischemic Limb/necrosis/gas	GPC±GNR± anaerobes	β-L-ase 1 or 2; group 1 or 2 carbapenem; second-/third-gen ceph + clindamycin or metronidazole
	MRSA risk factors	MRSA	Consider adding, or substituting with, glycopeptides; linezolid; daptomycin; fusidic acid T/S (±rif); doxycycline
	Risk factors for resistant GNR	ESBL	Carbapenems; FQ; aminoglycoside and colistin

Note: * Abbreviations: GPC, Gram-positive cocci (staphylococci and streptococci); GNR, Gram-negative rod; MRSA, methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum ß-lactamase-producing organism; S-S pen, semisynthetic penicillinase-resistant penicillin; ß-L-ase, ß-lactam, ß-lactamase inhibitor; ß-L-ase 1, amoxicillin/clavulanate, ampicillin/sulbactam; ß-L-ase 2, ticarcillin/clavulanate, piperacillin/tazobactam; doxy, doxycycline; group 1 carbapenem, ertapenem; group 2 carbapenem, imipenem, meropenem, doripenem; ceph, cephalosporin; gen, generation; Pip/tazo, piperacillin/tazobactam; FQ, fluoroquinolone with good activity against aerobic Gram-positive cocci (e.g., levofloxacin or moxifloxacin); cipro, antipseudomonal fluoroquinolone, e.g., ciprofloxacin, T/S, trimethoprim/sulfamethoxazole; rif, rifamp(ic)in. ** If some comorbidities such as azotemia, liver dysfunction, and obesity are present, adjusting the doses chosen for the patients should be considered

Currently, there are no tests able to determine the long-term resolution of osteomyelitis. The IWGDF proposes that a reduction in serum inflammatory markers, particularly ESR, and positive healing progression and radiological assessment can be useful when determining when to stop antibiotic therapy. Healthcare providers managing a DFU should consult with a surgical specialist in some situations such as severe infection or of moderate complicated infection, infection alongside widespread gangrene, necrotizing infection, suspicion of a deep abscess, compartment syndrome, or severe lower limb ischemia. If the bone is resected during surgery, a specimen of bone should be taken for culture to

Table 6.7 Characteristics indicative of serious diabetic foot infection and probable indications for hospitalization

A – Findings suggesting a more serious diabetic foot infection	
Wound	Penetrates to subcutaneous tissues (e.g., fascia, tendon, muscle, joint, or bone)
Cellulitis	Extensive (> 2 cm), distant from ulceration or rapidly progressive (including lymphangitis)
Local signs/ symptoms	Severe inflammation or induration, crepitus, bullae, discoloration, necrosis or gangrene, ecchymosis or petechia, and new anesthesia or localized pain
General	
Presentation	Acute onset/worsening or rapidly progressive
Systemic signs	Fever, chills, hypotension, confusion, and volume depletion
Laboratory tests	Leukocytosis, highly elevated C-reactive protein or erythrocyte sedimentation rate, severe or worsening hyperglycemia, acidosis, new/worsening azotemia, and electrolyte abnormalities
Complicating features	Presence of a foreign body (accidentally or surgically implanted), puncture wound, deep abscess, arterial or venous insufficiency, lymphedema, immunosuppressive illness or treatment, acute kidney injury
Failing treatment	Progression while on apparently appropriate antibiotic and supportive therapy
B – Some factors suggesting hospitalization may be necessary	
Severe infection (see findings suggesting a more serious diabetic foot infection above)	
Metabolic or hemodynamic instability	
Intravenous therapy needed (and not available/appropriate as an outpatient)	
Diagnostic tests needed that are not available as an outpatient	
Foot ischemia is present	
Surgical procedures (more than minor) required	
Failure of outpatient management	
Patient unable or unwilling to comply with outpatient-based treatment	
Need for more complex dressing changes than patient/caregivers can provide	
Need for careful, continuous observation	

detect remaining bone infection at the stump of the resected bone. It is reported that an aggressive surgical method with minor amputation reduces the risk of major amputation above the ankle as well as the length of hospitalization and associated costs [37, 98, 99]. Chronic osteomyelitis is correlated with a high rate of recurrence regardless of a long antibiotic therapy. The rate of infection recurrence is nearly 30%. Recurrence might be related to the partial resection of an infected bone or to resistant microorganisms remaining within their biofilm. Biofilms may shield pathogens from detection, and this is the reason for deep-tissue sampling. The recurrence of OM has to be taken into consideration in case of ulcer reappearance within 12 months after the first healing [38, 40, 100]. Infectious features of the diabetic foot are still uncertain because managing infectious characteristics of the diabetic foot is an extensive and challenging field, and the treatment of OM remains a highly debated topic of interest.

	MTP joint	PIP joint	DIP joint
Hammer toe	Hyperextended	Flexed	Extended
Mallet toe	Extended	Extended	Flexed
Claw toe	Hyperextended	Flexed	Flexed
Curly toe	Extended	Flexed	Flexed
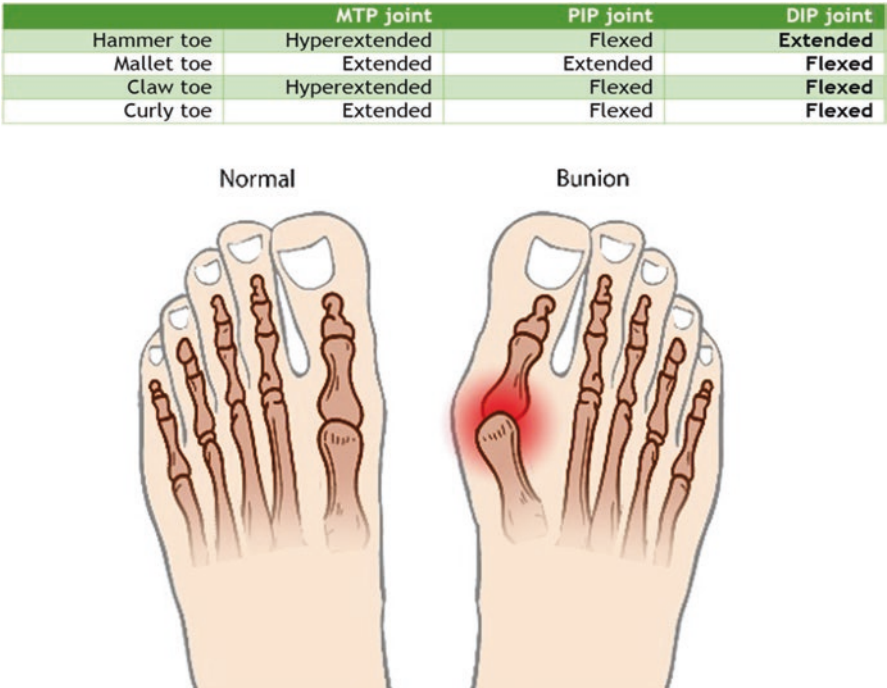
Fig. 6.4 Diabetic foot deformities

6.4   Diabetic Foot Deformity

Motor neuropathy results in weakness in the intrinsic muscles of the foot, consequently disturbing the balance between flexors and extensors of the toes. As a result, small muscle atrophy leads to metatarsophalangeal plantar flexion and the development of claw toes, hammer toes, prominent metatarsal heads, and pes cavus (Fig. 6.4). Repetitive pressure at the site of these deformities may result in tissue breakdown, callus formation, subsurface hemorrhage, and ulceration. Foot deformities (toe deformities and prominent metatarsal heads) are important contributing factors due to increased pressure and exposing the patient to increased risk of ulceration [101, 102].

6.4.1   Charcot Foot

Charcot foot or Charcot neuropathic arthropathy (CN) is an advanced, denervation-induced degeneration of the weight-bearing joints of the foot [103]. It is a severely difficult and a devastating complication for patients with peripheral neuropathy. Whereas it is most commonly correlated with diabetes, it may occur in any patient

with loss of afferent proprioceptive fibers. CN is present in nearly 10% of patients with diabetes, and diabetes is the most common factor of CN in the western world [104, 105].

Early identification and treatment former to the establishment of deformity is critical to improve outcomes. Clinical assessment and the radiographic appearance of the bones in the foot are the main issues to take into account during diagnosis. CN usually presents as a unilateral, localized, inflammatory reaction in a focal area of the foot or ankle with erythema, warmth, and swelling, possibly initiated by a trauma or repetitive microtrauma [106–108]. The involved bone undergoes changes during the stages of destruction including fragmentation and coalescence followed by consolidation, a process that may take months or even years to entirely resolve. CN is repeatedly misdiagnosed as cellulitis or osteomyelitis, which postpones the diagnosis and consequently leads to additional bony destruction. CN usually involves the tarsometatarsal (Lisfranc) joint, which is affected in about 50% of cases, but any joint in the foot or ankle can be involved. This leads to several fragmented bones, dislocations, and distorted anatomy [109–111].

After clinical examination, radiographs are obtained. Radiographs confirm the anatomic area affected (e.g., midfoot, ankle, etc.), but they also offer data about whether there is an urgent surgical indication (e.g., significant dislocation) that may need urgent consideration. If a neuropathic patient presents with foot erythema and edema after a recent injury, but with no wounds or remarkable radiographic changes, MR can demonstrate early stage disease. MR can also be beneficial for detecting infection or abscess formation [112, 113].

6.5 Diabetic Foot Ischemia

It is estimated that up to 50% of patients with diabetic foot ulceration have peripheral artery disease (PAD) in middle- and high-income countries [114, 115], while neuropathic ulcers are more prevalent in low-income countries [116, 117]. It is important to diagnose PAD in patients with DFU at the primary stage, because the existence of PAD is correlated with elevated risk of nonhealing ulcers, infection, amputation, and overall mortality. The prognosis of a patient with DFU, PAD, and amputation is worse than many common cancers, and the survival of up to 50% of these patients is less than 5 years [115, 118–120].

The risk factors of PAD include age ≥ 70 years, age 50–69 years with a history of diabetes and smoking, age 40–49 years with diabetes and another atherosclerosis risk factor, intermittent claudication or rest pain in feet, diminished pedal pulses, or atherosclerosis in another location (coronary, carotid, renal arteries, etc.). It is recommended to screen all diabetic patients for PAD annually [121–123]. Clinical examination such as pedal pulse palpitation does not exclude PAD in the majority of patients with diabetes and DFU. Therefore, evaluation of pedal Doppler arterial waveforms in combination with systolic ankle-brachial index (ABI) or toe-brachial index (TBI) measurement is necessary (Fig. 6.5). Of course, no definite modality
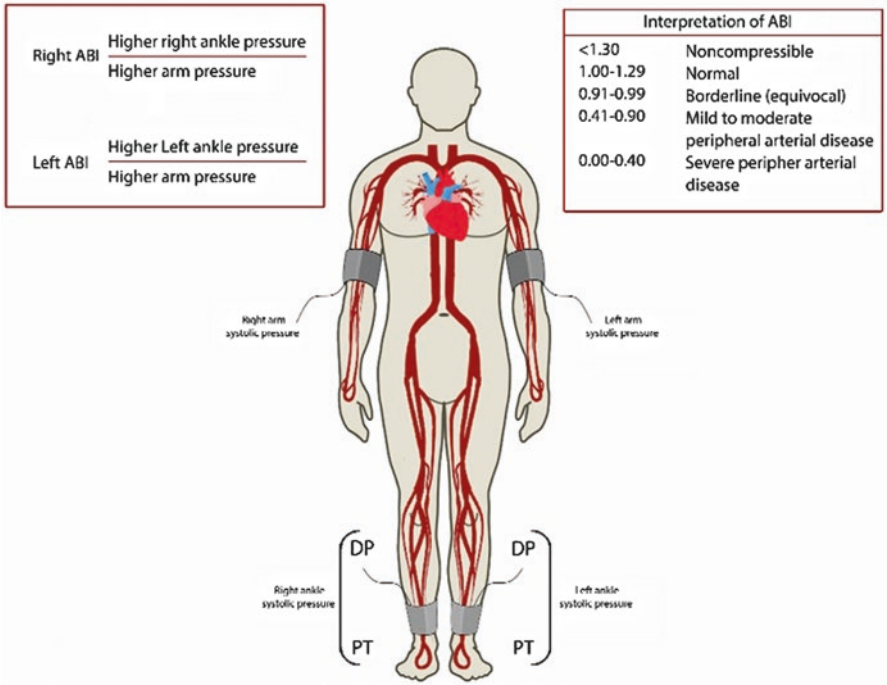
Fig. 6.5 Ankle-brachial index (ABI) measurement. [*DP* dorsalis pedis, *PT* posterior tibialis]

Table 6.8 Ischemia grading in PAD

Ischemia			
Grade	Ankle-brachial index	Ankle systolic pressure (mmHg)	Toe pressure, transcutaneous oxygen pressure (mmHg)
0	≥0.80	>100	≥60
1	0.6–0.79	70–100	40–59
2	0.4–0.59	50–70	30–39
3	≤0.39	<50	<30

has been shown to be ideal; however, PAD is less probable in the presence of an ABI 0.9–1.3, toe-brachial index ≥0.75, and triphasic pedal Doppler waveforms; but there is no exact value to exclude PAD reliably [21, 124, 125].

Vascular imaging in patients with a DFU should be taken into account, regardless of the bedside test findings, when the ulcer is not healed within 4–6 weeks even with standard care. Urgent vascular imaging and revascularization should always be considered an option in patients with a DFU and an ankle pressure < 50 mmHg, ABI < 0.5, a toe pressure < 30 mmHg, or a transcutaneous oxygen pressure (TcPO2) < 25 mmHg [126, 127] [Table 6.8]. It should be noted that reduced perfusion in a lower extremity is not just due to PAD because edema and infection can

cause a reduction in tissue oxygenation in the same way, and these should all be handled suitably [128, 129].

Any revascularization procedure should be part of a multidisciplinary care. Patients with a foot infection have a high risk of limb loss, and are considered a medical emergency. The 1-year major amputation rate for these patients is 44%, and postponing treatment can cause life-threatening sepsis [130]. When treating deep infections, such as a foot abscess that needs drainage to control the infection, immediate drainage should be undertaken first, followed by aggressive antibiotic therapy with the purpose of controlling sepsis because "time is tissue" in these patients. When the infection is controlled, arterial tree assessment should be considered. After blood flow has improved and the infection treated, a final operation may be needed with the aim of constructing a functional foot. In patients with severely impaired perfusion and severe tissue loss, but without infection, comprehensive debridement or amputation should not be performed until perfusion is repaired [131–133].

6.6 Basic Treatment

Diabetic foot ulcer treatment is based on the following items: revascularization, infection control, debridement, offloading, and dressing in addition to good glycemic control. Revascularization and infection control have already been discussed, and here we discuss these additional factors.

6.6.1 Debridement

Debridement is the removal of necrotic tissues or foreign-infected bodies from a wound, which is noted as the most important therapeutic step resulting in wound healing in DFU treatment. Debridement decreases bacterial counts, promotes production of local growth factors, and accelerates wound drainage. There are different types of debridement including surgical, enzymatic, autolytic, mechanical, and biological (Table 6.9). It is recommended to remove slough, necrotic tissue, and inclosing callus of a DFU with sharp debridement in preference to other methods [134–142].

The most important purpose of debridement is to change a chronic ulcer into an acute one. Furthermore, the more the debridement is repeated, the better the healing process. More than 90% of chronic wounds are complicated by biofilm. A biofilm can reform even after sharp debridement and can also postpone healing and recovery. Cadexomer iodine, a new-generation iodine formulation with microbead technology, can effectively handle biofilm along with exudate and has a de-sloughing function. An older debridement type that

Table 6.9 Different types of debridement in diabetic foot ulcer

Method	Explanation	Advantages	Disadvantages
Surgical or sharp	Callus and all nonviable soft tissues and bone remove from the open wound with a scalpel, tissue rippers, curettes, and curved scissors. Excision of necrotic tissues should extend as deeply and proximally as necessary until healthy, bleeding soft tissues and bone are encountered	Only requires sterile scissors or a scalpel, so is cost-effective	Requires a certain amount of skill to prevent enlarging the wound
Mechanical	This method includes wet to dry dressings, high pressure irrigation, pulsed lavage and hydrotherapy, and commonly used to clean wounds prior to surgical or sharp debridement	Allows removal of hardened necrosis	It is not discriminating and may remove granulating tissue; it may be painful for the patients
Autolytic	This method occurs naturally in a healthy, moist wound environment when arterial perfusion and venous drainage are maintained	It is cost-effective It is suitable for an extremely painful wound	It is time-consuming and may require an equivocal time for treatment
Enzymatic	The only formulation available in the United Kingdom contains Streptokinase and Streptodornase (Varidase Topical Wyeth Laboratories). This enzyme aggressively digests the proteins fibrin. Collagen and elastin, which are commonly found in the necrotic exudate of a wound	They can be applied directly into the necrotic area	Streptokinase can be systemically absorbed and is therefore contraindicated in patients at risk of an MI It is expensive
Biological	Sterile maggots of the green bottle fly (*Lucilia sericata*) are placed directly into the affected area and held in place by a close net dressing. The larvae have a ferocious appetite for necrotic material while actively avoiding newly formed healthy tissue	They discriminate between the necrotic and the granulating tissues	There may be a reluctance to use this treatment by patients and clinicians It is expensive

is classified as biological debridement is maggot debridement therapy (MDT) or larval therapy.

In this approach, sterile and live forms of the *Lucilia sericata* larvae are placed on the wound to produce a powerful autolytic enzyme that dissolves necrotic tissues, intensifies the healing procedures, and devastates bacterial biofilms. A number of studies have reported that MDT can significantly reduce wound odor and bacterial count, including MRSA [38, 143–148].

6.6.2 Offloading

Offloading techniques are known as pressure modulation and are believed as the most important part of neuropathic ulcer management in patients with diabetes. These devices spread plantar pressure and handle extreme plantar tissue stress with the aim of healing and preventing. DFU offloading has to be accompanied with revascularization, infection control, dressing, debridement, and metabolic control, for each of these components to be effective. If the patient is not off-loaded, none of these components will result in wound healing, or the period of healing may increase [33, 149, 150]. Various offloading modalities are in use at present [Table 6.10]. The choice of these approaches is defined by the patient's physical characteristics and abilities to adhere to the treatment in conjunction with the location and severity of the ulcer. Gait function worsening with no opportune intervention among patients with a DFU may lead to severe undesirable outcomes, including a deteriorating DFU, amputation, early weakness, risk of falling, and deficiency of independency, which may exacerbate their conditions more [33, 150].

The most applicable offloading technique for the treatment of a neuropathic DFU is a total contact cast (TCC) [150–152]. The TCC is padded and formed carefully to the contour of the foot with a heel included for walking (Fig. 6.6). The cast is designed to reduce pressure from the ulcer and disseminate pressure over the whole foot surface. Furthermore, the patient is incapable of removing the cast, which increases compliance, decreases activity levels, and as a result progresses wound healing [150, 153].

However, a TCC does not allow for daily assessment of the wound, which is frequently contraindicative in soft tissue or bone infections [137, 154, 155]. In particular cases, it is recommended to apply other types of offloading techniques for instance a removable cast walker (RCW) (Fig. 6.6) or instant TCC (iTCC). Forefoot and hindfoot offloading with half shoes is mainly practical in DFU management

Table 6.10 Different types of offloading techniques [150]

Technique	Casting techniques	Footwear-related techniques	Surgical offloading techniques	Other techniques
Examples	TCC (Fig. 6.6) iTCC RCW (Fig. 6.6) Scotch-cast boots Windowed easts Custom splints	Shoes or half shoes (Fig. 6.7) Sandals Insoles In-shoe orthoses Socks	ATL Liquid silicone injections/tissue augmentation Callus debridement Metatarsal head resection osteotomy/ arthroplasty/ ostectomy/exostectomy External fixation	Bed rest Crutches/canes/ wheelchairs Bracing (patella tendon bearing ankle-foot orthoses) Walkers Offloading dressings Felted foam/padding Plugs

Abbreviations: *TCC* total contact cast, *iTCC* instant TCC, *RCW* removable cast walkers, *ATL* Achilles tendon lengthening
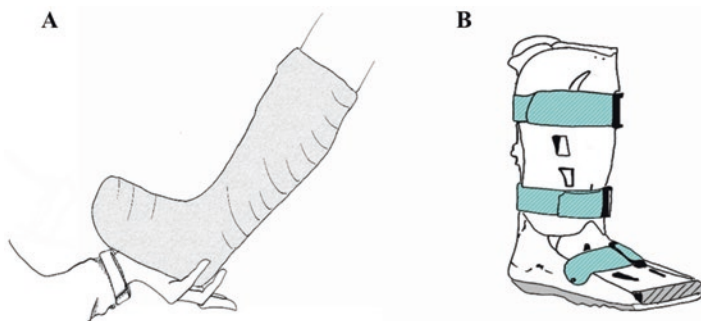
Fig. 6.6 (**a**) Total contact casts (TCC) and (**b**) removable cast walker (RCW)

(Fig. 6.7). After healing, patients should wear any footwear or insole that does not cause ulcer formation, as footwear can play an important role. Additionally, there are modern methods such as foot scanners able to measure peak plantar pressure which is essential when making shoes designed according to the weight-bearing positions in the feet [156, 157].

6.6.3   Advanced Dressings

Novel dressings have been the main breakthrough for DFU management over the last decades [158, 159]. Preferably, dressings should allow moisture balance, growth factor stimulation, protease sequestration, oxygen permeability, antimicrobial activity, and promotion of autolytic debridement that accelerates granulation tissue formation and the reepithelialization process. Moreover, they should have high effectiveness and persistent time of effect [158, 160]. However, there is no definite dressing type that satisfies all of the needs of a patient with a diabetic foot ulcer. The choice of the dressing is defined according to the DFU reasons, wound site, depth, exudates, wound margins, presence of infection or pain, and need for adhesiveness [159]. The most important types of dressings used for DFU are films, hydrogels, hydrocolloids, alginates, foams, and silver-impregnated dressings (Table 6.11). Selection of dressings should also be based on the exudate control, ease of using, and cost. Dressings are selected and applied in accordance with DFU features; nevertheless, hydrogels are the most accepted kind of dressing for all DFU types. Some dressings have agents with antimicrobial materials (honey, iodine, silver, polyhexamethylene) and some materials to modify the biology of the chronic wound (affecting surface protease activity). It is recommended to consider the use of placental-derived products as an adjunctive treatment besides the best standard of care because human placental membranes have growth factors, collagen-rich extracellular matrix, and cells including mesenchymal stem cells, neonatal fibroblasts, and epithelial cells that prepare the essential procedures to manage wound healing [15, 139].

Fig. 6.7 Half shoe for
forefoot offloading
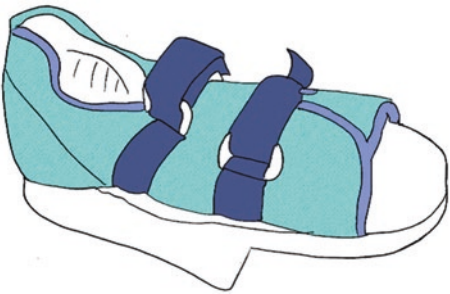
Table 6.11 Selection of the type of dressing based on DFU features

Wounds				
Necrotic	Sloughy	Exudate	Granulating	Epitheliasing
Hydrogel	Hydrogel	Foam	Hydrocolloid	Hydrocolloid
Hydrocolloid	Hydrocolloid	Alginate	Foam	Foam
	Foam			Non-adherent dressing
	Alginate			
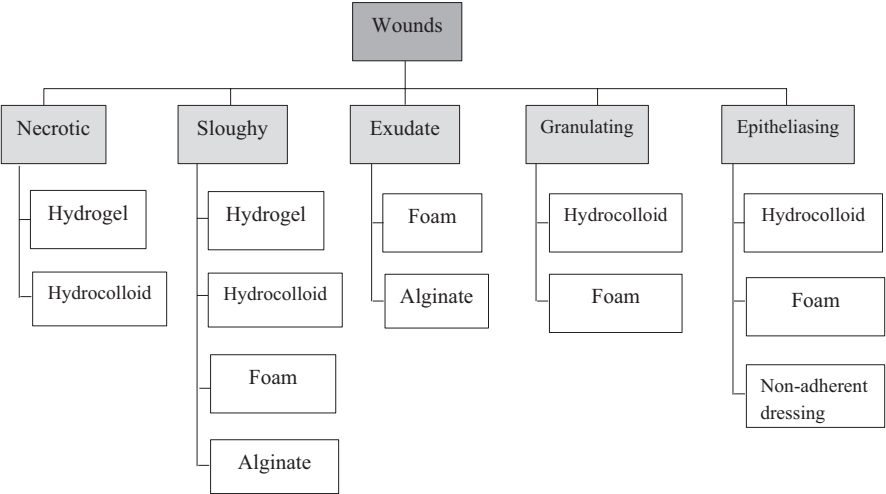
6.7 Novel Treatment Strategies

In this section, a number of novel treatment strategies for the management of DFUs
and improvement of the healing process are presented.

6.7.1 Hyperbaric Oxygen Therapy (HBOT)

This technique has been demonstrated to be advantageous in DFU treatment. It is
considered that intermittent administration of 100% oxygen usually in daily ses-
sions is useful. The patient breathes pure oxygen at 1.4–3.0 entire atmospheres dur-
ing 3 periods of 30 min (overall 90 min) intercalated by 5-min intervals in a
hyperbaric chamber during each session [161–163] (Fig. 6.8). Some studies have
demonstrated that HBOT improved wound tissue hypoxia, decreased edema,

Fig. 6.8 Hyperbaric chamber
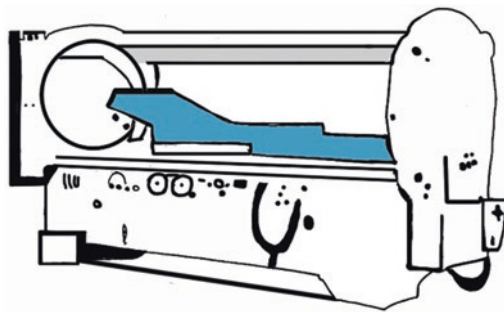
increased perfusion, downregulated inflammatory cytokines, and stimulated fibroblast proliferation, collagen construction, and angiogenesis [164–166]. However, adjuvant use of this technique in DFU has raised controversial concerns. HBOT does not replace the need for antibiotic therapy or surgical wound debridement. In addition, HBOT is offered in just a few societies because of its high cost and is a time-consuming method [17, 139, 167].

6.7.2 Negative Pressure Wound Therapy

Negative pressure wound therapy (NPWT) or vacuum therapy is a noninvasive wound resolution procedure that uses supervised and limited negative pressure to promote healing in chronic and acute wounds. This method applies a sterile and latex-free polyurethane or polyvinyl alcohol foam dressing prepared for each wound, protected with an impermeable adhesive cover. Often, 80–125 mmHg of negative pressure is utilized in cycles or constantly. Fluid is extracted from the wound using a pumping mechanism [139, 168, 169].

NPWT detaches edema and exudate, decreases bacterial colonization, develops reconstruction of blood vessels and granulation tissue formation, and increases wound oxygenation and contraction. Probable adverse effects have been reported and include wound maceration, retention of dressings, and possible wound infection [139, 170–172].

6.7.3 Bioengineered Skin

During the last decades, bioengineered skin (BES) has been used as a new therapeutic technique to treat DFU. This method substitutes the damaged and deteriorating native extracellular matrix (ECM) with a new ground material matrix that initiates a new healing route with cellular elements. Currently, three kinds of BES products are approved in the United States for use with DFUs: Derma graft (Advanced

BioHealing Inc., La Jolla, CA), Apligraf (Organogenesis Inc., Canton, MA), and, more recently, Oasis (Cook Biotech, West Lafayette, IN) [172–175].

BES products contain cells that have been cultured in vitro, expediting DFU healing by active secretion of growth factors. Additionally, BES may provide the cellular substrate and the necessary molecular factors that augment angiogenesis and subsequently improve wound healing. Peripheral ischemia, one of the challenging features of DFU, is a serious contributing factor that influences BES transplantation. Consequently, surgical revascularization, decompression, and wound bed preparation are the known necessary components for BES therapy. Additionally, since this method requires infection control, all these requirements may cause high costs and require long-term care [175–178].

6.7.4 Platelet-Rich Plasma

The use of autologous platelet-rich plasma (PRP) has emerged as an adjunctive approach for DFU treatment in recent years. PRP is obtained from centrifugation of whole blood, which is divided into three layers: platelet-poor plasma, platelet-rich plasma, and red blood cells. These platelets contain a number of active proteins that aid in the biological route of wound healing [179, 180]. Platelet alpha-granules release molecules including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), fibrinogen, fibronectin, and vitronectin. Among these, recombinant human PDGF (rhPDGF) (Becaplermin or Regranex), which is a hydrogel including 0.01% of PDGF-BB (rhPDGF-BB), has shown enhanced healing rates in comparison with controls in several clinical trials and earned Food and Drug Administration (FDA) approval [165, 181]. Even with FDA approval, the clinical application of Becaplermin is limited due to its high price and the possibility of carcinogenesis following its topical administration. Also, platelet delta-granules release serotonin, histamine, dopamine, calcium, and adenosine, which regulate wound healing. To date, small randomized controlled studies and case reports have evaluated the outcomes of topical autologous PRP on DFU healing. Many confounding variables are relevant to PRP use, so there is still a considerable challenge in making standardized protocols for patient use [180, 181].

6.7.5 Physical Therapy

Physical therapy includes shockwaves, ultrasound, laser therapy, magnetism, and electrical current stimulation. Electrical stimulation (ES) is an adjunctive therapy for DFU healing. At the present time, there is a substantial body of work that supports the effectiveness of ES for DFU healing. It is recommended that ES could recover reduced blood flow, infection, and inadequate cellular responses. This

method is a low-priced and uncomplicated intervention to improve the healing process in DFU [182–183].

Low-level laser therapy (LLLT) or soft laser is known to provide direct light energy to body cells. The technology of low-energy laser was established more than three decades ago in medicine; however, it has not received much consideration. The absorbed laser energy motivates molecules and atoms of cells but does not result in a large rise in tissue temperature. It has a stimulating influence on cell mitosis, keratinocyte passage and proliferation, and cytokine construction, and may result in elevated dermal angiogenesis [184–186]. Various laser wavelengths are able to penetrate human tissue to different depths. Low-energy laser radiation has been discovered to have a stimulating outcome on cells, while high-energy radiation has an inhibiting effect. It is recommended to apply lasers as adjuvant therapy to stimulate wound healing in nonhealing ulcers [185, 186].

6.7.6   Ozone Therapy

Ozone is a gas formulated of three atoms of oxygen within a cyclic arrangement. The use of ozone (O_3) within medicine was initiated in the mid-nineteenth century. This gas can be used to treat several diseases because of its antioxidant and antibacterial properties. For example, it is useful in the treatment of chronic infections caused predominantly by antibiotic-resistant pathogens [187, 188]. There is increasing evidence that O_3 can be applied to treat DFU. Moreover, O_2-O_3 treatment can increase VEGF, TGF-β, and PDGF levels and repair localized gangrene. Fibroblast proliferation promotion by O_3 improves remodeling of the intercellular matrix and healing of the area around the DFU [188, 189]. Some studies report that the enhanced rate of wound closure may be as a result of O_2 tension by O_3 in the enclosing wound area that operates as an antibacterial material to reduce bacterial infection. A team of German scientists used O_3 treatment for diabetic skin ulcers in the late twentieth century [190]. They used a polythene bag for approximately 25 minutes with a concentration ranging from 10 to 80 µg/mL. Offloading and debridement of the DFU is a basic stage in wound therapy for neuropathic ulceration. A combination of O_2-O_3 therapy can be helpful in nonhealing wounds. O_2-O_3 is known as an antiseptic as it deactivates bacteria by breaking their cover through oxidation of specific proteins and lipids. In addition, interferon, TNF, and IL-2 stimulate the immune system, leading to a reduction of infection by O_3 therapy in DFU treatment. Although O_3 therapy is considered safe and without adverse effects, it may be toxic if used outside its therapeutic dose. Intralesional O_3 injection for DFU treatment has not been used in any study because its safety has not yet been determined. Furthermore, O_3 therapy is not endorsed for deep, severely infected, or necrotic wounds [187, 189].

6.7.7 Cold Atmospheric Plasma Therapy

Plasma is a shape of matter other than solids, liquids, or gases; hence, it is mentioned as the fourth state of matter. It can be industrially produced by using high voltages to narrow gas-filled gaps which cause strong electrical fields. A method of producing plasma at atmospheric pressure is through use of dielectric-barrier discharge (DBD) that reduces current flow and gas warming. Charged fragments, chemically reactive species (O_3, OH, H_2O_2, O, NxOy, etc.), ultraviolet radiation (UV-A and UV-B), and great fluctuating electric fields in addition to weak electric currents are produced by DBD in air. Regular plasmas used for sterilizing medical devices or for tissue cauterization are both thermal plasmas [191, 192]. As an alternative to thermal plasma, cold atmospheric plasma (CAP) is available, which is also known as atmospheric cold plasma (ACP), cold atmospheric pressure plasma (APP), or tissue-tolerable plasma (TTP). CAP has demonstrated its advantages in reducing many fungi and bacteria including antibiotic-resistant biofilm-forming strains not only in vitro but also through use of in vivo models. Wound healing was shown to increase through the upregulation of IL-6, IL-8, MCP-1, TGF-β 1/2, and collagen type I in vivo. Studies have reported that CAP treatment for 3 min daily increased diabetic wound healing through inflammation inhibition, oxidative stress reduction, and angiogenesis enhancement without toxicity to the liver and kidney. Nevertheless, the CAP effects on wound healing are still unclear [192, 193].

6.7.8 Stem Cells

Along with the arrival of regenerative medicine, stem cell-based therapies have become the focus of scholarly attention. Stem cell therapy, particularly mesenchymal stem cell therapy, is considered as a therapeutic option for diabetic foot with ischemic arterial limb disease, administered via multiple intramuscular injections. Although there is no exact cure for diabetic ulcers to date, there is an immense possibility to discover a definite way for treating them using stem cells [194, 195].

6.8 Other Lower Limb Infections in Diabetes

Bedridden patients with a DFU may develop spreading of infection alongside the flexor tendons to the calf because pus transfers to the proximal end of the central plantar zone, in the direction of the region of the medial malleolus. Foot compartments are known as medial, central, lateral, and the interosseous compartment, whereas the leg is divided into three compartments anterior, lateral, and posterior by intermuscular septa, jointly with the interosseous membrane. The compartments in the foot are connected to their matching parts in the leg within tunnellike spaces

around the tendons that pass through both the leg and the foot. The medial foot compartment is connected to the posterior leg compartment by means of flexor hallucis longus tendon. The central foot compartment connects to the posterior leg compartment by means of the flexor hallucis longus tendon and flexor digitorum longus tendon. This facilitates the spread of infection from the foot to the leg. Diabetic foot infection expanding to the leg is a common clinical problem. Several risk factors related to the extension of infection to the leg were assessed in a retrospective case-control study; and each of these clinical factors can be evaluated easily on the patient's first assessment. Toe amputation, wound localized on the heel, wound size more than 5 cm as well as advanced Wagner grade 3–5, and severe sepsis grade 4 may be regarded as risk factors for spreading of an infection to the leg in patients with a DFU. Nevertheless, this spread will not cause a poor prognosis to the final outcome if sufficient treatment is provided [196]. In accordance with some studies, no relationship exists between extension of infection to the leg and peripheral vascular disease (PVD), which, in view of this fact, the existence of ischemia and infection together will increase the risk of a major amputation before the extension of infection to the leg will happen. Admittedly, it is helpful to differentiate leg ulceration from ulcers restricted to the foot, in order to emphasize the differences in the prevalence of ulceration causing factors at these locations. Venous disease, arterial disease, and diabetes are etiological factors in isolated ulceration of the foot as opposed to leg ulceration [197]. Moreover, numerous studies have shown that diabetes is an independent risk factor for poor postoperative outcomes such as mortality and surgical site infection, in patients undergoing a wide range of surgical interventions [198]. Diabetes is a routine comorbidity in patients experiencing joint replacement surgery. Because diabetes is associated with a number of micro- and macrovascular complications, and might have an impact on bone remodeling, total hip replacement (THR) in diabetic patients may incur severe medical complications. According to studies conducted on a large number of THR and total knee replacement patients, the risk of postoperative complications is increased in patients with diabetes. For example, after joint replacement surgery, a higher rate of complications including pneumonia and joint infection may occur in patients with diabetes [199–201]. The current evidence on the rate and extent of postoperative complications in diabetic patients following joint replacement surgery is inadequate. Any kind of tissue damage, such as surgery during THR, leads to insulin resistance and hyperglycemia, and postoperative hyperglycemia has been associated with an elevated risk of surgical site infection. The same pathophysiology is also present after coronary artery bypass graft and healing in the leg [202]. Diabetic patients must take their vein health into special consideration. In fact, about one-third of diabetic patients with chronic venous insufficiency will also develop venous ulcerations before the age of 40 as a consequence of the loss of circulation and sensation in diabetes particularly in peripheral neuropathy [197, 203].

Chronic leg ulcers involve more than 1% of the population. It is advantageous to predict outcome by detecting the contributing factors in order to better manage their prognosis [203]. A survey that investigated the etiological factors that contribute to leg ulcers included obesity, diabetes, female gender, anemia, age, and peripheral

vascular disease. Also leg wound complications described as hematoma, cellulitis, necrosis, dehiscence, and abscess were reported more frequently in patients with diabetes than nondiabetics [204].

6.9 COVID-19 Pandemic and Diabetic Limb

At the time of writing this chapter, people are experiencing a new and widespread pandemic known as COVID-19 all over the world. Several studies have reported a greater risk of COVID-19 in patients with diabetes. Furthermore, as a DFU is the most common cause of hospitalization in diabetic individuals, it seems logical to make an effort to reduce hospital-related COVID-19 transmission. This requires reducing interaction between staff, patients, and equipment. In the course of the COVID-19 pandemic, inhibition and management of nosocomial infection is more critical [205, 206].

Patients with a DFU may have fever and usually present with local redness, swelling, pain, inflammatory secretions, and consequently increased inflammatory response markers [e.g., WBC, CRP, ESR, and/or procalcitonin], which largely overlap with signs and symptoms of COVID-19. If the patient has a confirmed or suspected positive diagnostic criterion for COVID-19, they should be transferred to a designated hospital immediately. Glycemic control is critical in diabetic patients with COVID-19, as patients with poor glycemic control are more predisposed to complications, and increased mortality is expected. Blood glucose monitoring is necessary, and it is recommended to keep blood glucose levels under control by insulin therapy. Elective surgery (e.g., vacuum drainage, transverse tibial bone transport technique, and arterial revascularization) should be procrastinated in the short term. In partial surgery (such as interventional treatment, conservative treatment, or amputation), procedures can be postponed if these delays do not influence the recent condition of the patient. For patients who need emergency surgery (e.g., debridement or local decompression), the procedure can be done after consultation with surgeons, anesthesiologists, and other related specialties by observing protective measures [206].

In patients with infected DFUs such as local abscesses, a severe inflammatory response, or septic shock, drainage and control of systemic infection is urgent. Patients with progressive infection, imposed to liver and kidney dysfunction, septic shock, and failure of nonsurgical treatment, who need emergency amputation surgery as a lifesaving procedure, have to undergo surgery urgently. In patients with COVID-19, the severity of pneumonia has to be considered at the time of selecting treatment options. Percutaneous transluminal angioplasty (PCTA) is appropriate for patients with mild and moderate COVID-19. The risks and benefits of surgery need to be thoroughly assessed prior to selection of surgical treatment.

The diagnosis and treatment approaches explained above should be in accordance with receiving timely and acceptable treatment in conjunction with efficient COVID-19 prevention measures in patients [206].

Implementing a triage system for DFU, in-home visits, higher protected office visits, telemedicine, and remote patient monitoring can help healthcare providers deal with patients to reduce the COVID-19 risk. The aim within the pandemic is to decrease the burden on the healthcare system by means of preserving patients with a DFU, protecting them, and keeping them functional at home. Wound centers which undertake DFU care should shut down their services or reduce their work time during pandemics. DFU surgeries and interventions may be incorrectly categorized as nonessential, so these patients are at risk of fast infection development, which may cause increased rate of amputations and deaths. Additionally, patients with diabetes are a population with high chance of mortality from COVID-19. The podiatrist's proposed triage system for lower-extremity wounds and diabetic foot problems is demonstrated in Table 6.12.

Patients who need revascularization can be managed in office-based labs by a vascular surgeon, a cardiologist, or an interventional radiologist in outpatient service.

In telemedicine and remote approaches, some methods facilitate this process to screen for infection and wound progress assessment. These include FaceTime and Google Glass in wound-based assessment, combinations of "store and forward" photos, short message service (SMS) text, or text video chat [208]. Increased cytokine levels (IL-6, IL-10, and tumor necrosis factor (TNF)-α) are key components in adverse COVID-19 outcomes. Simultaneously, cytokine variations and increases

Table 6.12 The podiatrist's proposed triage system for lower-extremity wounds and diabetic foot problems [207]

	Conditions	Site of care	Urgency
Critical (0.25% of patients with diabetes)	– IDSA severe and some moderate infections – Gas gangrene – SRS/sepsis – Acute limb-threatening ischemia	Hospital	Priority 1 Urgent

Serious (0.75% of patients with diabetes)	– IDSA Mad and some moderate infections (including osteomyelitis)
	  – Chronic limb-threatening ischemia (CLTI)
	  – Dry gangrene
	  – Worsening foot ulcers
	  – Active Charcot foot
		Outpatient clinic Office-based lab Surgery center Podiatrist office	Priority 2

Guarded (3% of patients with diabetes)	– Improving foot ulcer – Inactive Charcot foot (not yet in stable footwear)	Podiatrist office Home Telemedicine	Priority 3
Stable (94% of patients with diabetes)	– Uncomplicated venous foot ulcer – Recently healed foot ulcer – Inactive Charcot foot (in stable footwear) – Healed amputation	Home Telemedicine	Priority 4

are observed in patients with a DFU. An imbalance in pro-inflammatory cytokine release has been associated with the pathogenesis of Charcot osteoarthropathy [205]. Additionally, neuropathy principally contributes to DFU development, and at the same time, its severe form may decrease the inflammatory response to infections in patients with DFU and have some influence on the pro-inflammatory cytokine production in the event of a COVID-19 infection [118]. Also, ischemia as a result of PAD is another important contributing factor to a DFU [29]. In severe ischemia, clinicians are worried about intravenous antibiotics not reaching lower-extremity infections sites. Accordingly, there is some anxiety about how intravenous drugs used in COVID-19 infection may reach their target sites in DFU patients. In contrast, COVID-19 patients with dyspnea normally lessen their daily activities (or this may be due to quarantine), which is valuable in off-loading the DFU and may lead to advanced healing rates of neuropathic ulcers [205].

The question posed here is whether there is any potential relationship between COVID-19 and the diabetic foot regarding these points. It is just a hypothesis at this juncture, and more knowledge is required to prove or disprove it.

6.10   Conclusions

In conclusion, diabetic foot management is a multidisciplinary approach and needs a well-functioning teamwork of general physicians, and endocrinologists, as well as specialists in infectious disease, vascular surgery, orthopedics, interventionl radiology, orthotics, prosthesis and educated nurses. The following issues need to be taken into account in order to improve the outcome and prognosis: patient education (not only in treatment but also in the prevention of a DFU is a key component), glycemic control (HbA1c $\leq$ 7% ideally), sufficient debridement, suitable offloading, advanced selection of dressings, and appropriate footwear. Diabetic foot rehabilitation has to be utilized after amputation.

References

1. Zhang H, Qi D, Gu H, Wang T, et al. Trends in the prevalence, awareness, treatment and control of diabetes in rural areas of northern China from 1992 to 2011. J Diabetes Investig 2020; 11: 241–249. https://doi.org/10.1111/jdi.13095
2. Ramachandran A, Snehalatha C, Shetty AS, Nanditha A. Trends in prevalence of diabetes in Asian countries. World J Diabetes 2012; 3: 110–117. https://doi.org/10.4239/wjd.v3.i6.110
3. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011and 2030. Diabetes Res Clin Pract 2011; 94: 311–321. https://doi.org/10.1016/j.diabres.2011.10.029
4. Shahbazian H, Yazdanpanah L, Latifi SM. Risk assessment of patients with diabetes for foot ulcers according to risk classification consensus of international Working Group on Diabetic Foot (IWGDF). Pak J Med Sci 2013; 29: 730–734. https://doi.org/10.12669/pjms.293.3473

5. Marshall SM. A life course perspective on diabetes: developmental origins and beyond. Diabetologia 2019; 62:1737–1739. https://doi.org/10.1007/s00125-019-4954-6

6. Paola LD, Cimaglia P, Carone A, Scavone G, et al. Limb salvage in diabetic patients with no-option critical limb ischemia: outcomes of a specialized center experience. Diabetic Foot & Ankle 2019, 10(1): 1696012. https://doi.org/10.1080/2000625X.2019.1696012

7. Leone S, Pascale R, Vitale M, Esposito S. Epidemiology of diabetic foot. Infez Med 2012; 20 (1): 8–13.

8. Richard JL, Schuldiner S. Epidemiology of diabetic foot problems. Rev Med Interne 2008; 29 (2): S222-S230. https://doi.org/10.1016/S0248-8663(08)73949-3.

9. Ragnarson Tennvall G, Apelqvist J. Health-economic consequences of diabetic foot lesions. Clin Infect Dis 2004; 39(2): S132-S139. https://doi.org/10.1086/383275.

10. Yazdanpanah L, Shahbazian H, Nazari I, Arti HR, et al. Prevalence and related risk factors of diabetic foot ulcer in Ahvaz, south west of Iran, Diab Met Syndr: Clin Res Rev (2018). https://doi.org/10.1016/j.dsx.2018.03.018.

11. Sen CK. Human wounds and its burden: An updated compendium of estimates. Advances in Wound Care 2019; 8(2):39–48. https://doi.org/10.1089/wound.2019.0946

12. Yazdanpanah L, Shahbazian H, Nazari I, Arti HR, et al. Incidence and risk factors of diabetic foot ulcer: A population-based diabetic foot cohort (ADFC Study)—Two-year follow-up study. International Journal of Endocrinology 2018: Article ID 7631659, 9 pages. https://doi.org/10.1155/2018/7631659

13. Bortoletto MS, Andrade SM, Matsuo T, Haddad M C, et al. Risk factors for foot ulcers – A cross sectional survey from a primary care setting in Brazil. Prim Care Diabetes 2014; 8: 71–76. https://doi.org/10.1016/j.pcd.2013.04.003

14. Waaijman R, Haart M, Arts ML, Wever D, et al. Risk factors for plantar foot ulcer recurrence in neuropathic diabetic patients. Diabetes Care 2014; 37: 1697–1705. https://doi.org/10.2337/dc13-2470

15. Monteiro-Soares M, Boyko EJ, Ribeiro J, Ribeiro I, et al. Predictive factors for diabetic foot ulceration: A systematic review. Diabetes Metab Res Rev 2012; 28: 574–600. https://doi.org/10.1002/dmrr.2319.

16. Wu L, Hou Q, Zhou Q, Peng F. Prevalence of risk factors for diabetic foot complications in a Chinese tertiary hospital. Int J Clin Exp Med 2015; 8(3):3785–3792.

17. Alavi A, Sibbald RG, Mayer D, Goodman L, et al. Diabetic foot ulcers: Part II. Management. *J Am Acad Dermatol* 2014; 70: 21.e1-2124; quiz 21.e1-2124 https://doi.org/10.1016/j.jaad.2013.07.048.

18. Anichini R, Brocco E, Caravaggi CM, Da Ros R, et al. Physician experts in diabetes are natural team leaders for managing diabetic patients with foot complications. A position statement from the Italian diabetic foot study group. Nutrition, Metabolism and Cardiovascular Diseases 2020; 30(2):167–178. https://doi.org/10.1016/j.numecd.2019.11.009

19. Formosa C, Gatt A, Chockalingam N. Diabetic foot complications in Malta: prevalence of risk factors. Foot (Edinb) 2012; 22: 294–297. https://doi.org/10.1016/j.foot.2012.08.008.

20. Bus SA, van Deursen RW, Armstrong DG, Lewis JEA, et al. Footwear and offloading interventions to prevent and heal foot ulcers and reduce plantar pressure in patients with diabetes: a systematic review. Diabetes Metab Res Rev 2015; 32(s1): 99–118. https://doi.org/10.1002/dmrr.2702.

21. Schaper NC, Van Netten JJ, Apelqvist J, Bus SA, et al. IWGDF guidelines on the prevention and management of diabetic foot disease. Available on: https://iwgdfguidelines.org/guidelines/guidelines/

22. Pérez-Panero AJ, Ruiz-Muñoz M, Cuesta-Vargas AI ,Gónzalez-Sánchez M. Prevention, assessment, diagnosis and management of diabetic foot based on clinical practice guidelines: A systematic review. Medicine 2019; 98:35(e16877). https://doi.org/10.1097/MD.0000000000016877.

23. Wennberg L, Widgren S, Axelsson R, Gerok-Andersson R, et al. Multidisciplinary diabetic foot care in Sweden – A national survey. Diabetes research and clinical practice 2019; 149:126–131. https://doi.org/10.1016/j.diabres.2019.02.003

24. Jiao F, Cheung Fung CS, Fai Wan EY, Chun Chan AK, et al. Five-year cost-effectiveness of the multidisciplinary Risk Assessment and Management Programme–Diabetes Mellitus (RAMP-DM). Diabetes Care 2018; 41:250–257. https://doi.org/10.2337/dc17-1149.

25. Jiao F, Fai Wan EY, Cheung Fung CS, Chun Chan AK, et al. Cost-effectiveness of a primary care multidisciplinary Risk Assessment and Management Program for patients with diabetes mellitus (RAMP-DM) over lifetime. Endocrine 2019; 63:259–269. https://doi.org/10.1007/s12020-018-1727-9

26. Wang C, Mai L, Yang C, Liu D, et al. Reducing major lower extremity amputations after the introduction of a multidisciplinary team in patient with diabetes foot ulcer. BMC Endocrine Disorders 2016; 16:38. https://doi.org/10.1186/s12902-016-0111-0

27. Karki DB, Yadava SK, Pant S, Thusa N, et al. Prevalence of sensory neuropathy in Type 2 diabetes mellitus and its correlation with duration of disease. Kathmandu Univ Med J 2016; 54(2):120–124.

28. Ziegler D, Rathmann W, Dickhaus T, Meisinger C, et al. Prevalence of polyneuropathy in pre-diabetes and diabetes is associated with abdominal obesity and macro-angiopathy. Diabetes Care 2008; 31:464–469.

29. Ziegle I, Papanas N, Vinik AI, Shaw JE. Handbook of Clinical Neurology; Chapter 1 – Epidemiology of polyneuropathy in diabetes and pre-diabetes, Handb Clin Neurol. 2014, 126: 3–22. https://doi.org/10.1016/B978-0-444-53480-4.00001-1Getrightsandcontent

30. Breiner A, Lovblom LE, Perkins BA, Bril V. Does the prevailing hypothesis that small-fiber dysfunction precedes large-fiber dysfunction apply to Type 1 diabetic patients? Diabetes Care 2014; 37:1418–1424. https://doi.org/10.2337/dc13-2005.

31. Tabatabaei-Malazy O, Mohajeri-Tehrani MR, Madani SP, Heshmat R, et al. The prevalence of diabetic peripheral neuropathy and related factors. Iranian J Publ Health 2011; 40(3): 55–62.

32. Sveen KA, Karimé B, Jørum E, Mellgren SI, et al. Small-and large-fiber neuropathy after 40 years of type 1 diabetes. Diabetes Care 2013, 36:3712–3717.

33. Ling E, Lepow B, Zhou H , Enriquez A, et al. The impact of diabetic foot ulcers and unilateral offloading footwear on gait in people with diabetes. Clinical Biomechanics 2020; 73:157–161. https://doi.org/10.1016/j.clinbiomech.2020.01.014.

34. Raspovic KM, Wukich DK. Self-reported quality of life and diabetic foot infections. J Foot Ankle Surg 2014; 53:716–719.

35. Lavery LA, Armstrong DG, Murdoch DP, Peters EJ, Lipsky BA. Validation of the infectious diseases society of America's diabetic foot infection classification system. Clin Infect Dis 2007; 44:562–5.

36. Ndosi M, Wright-Hughes A, Brown S, et al. Prognosis of the infected diabetic foot ulcer: A 12-month prospective observational study. Diabet Med 2018; 35:78–88.

37. Giurato L, Meloni M, Izzo V, Uccioli L. Osteomyelitis in diabetic foot: A comprehensive overview. World J Diabetes 2017; 8(4): 120–171.

38. Tan TW, Shih CD, Concha-Moore KC, et al. Disparities in outcomes of patients admitted with diabetic foot infections. PLoS One 2019; 14:e0211481.

39. Jia L, Parker CN, Parker TJ, Kinnear EM, et al. Incidence and risk factors for developing infection in patients presenting with uninfected diabetic foot ulcers. PLoS ONE 2017; 12(5):e0177916. https://doi.org/10.1371/journal.pone.0177916

40. Barwell ND, Devers MC, Kennon B, Hopkinson HE, et al. Diabetic foot infection: Antibiotic therapy and good practice recommendations. Int J Clin Pract 2017; 71:e13006. https://doi.org/10.1111/ijcp.13006.

41. Zha ML, Cai JY, Chen HL. A bibliometric analysis of global research production pertaining to diabetic foot ulcers in the past ten years. J Foot Ankle Surg 2019; 58:253–9.

42. Paisley AN, Kalavalapalli S, Subudhi CP, Chadwick PR, et al. Real time presence of a microbiologist in a multidisciplinary diabetes foot clinic. Diabetes Res Clin Pract 2012; 96:e1–3.

43. Frykberg RG. An evidence-based approach to diabetic foot infections. The American journal of surgery 2003; 186(5):44–54.
44. Grigoropoulou P, Eleftheriadou I, Jude E.B, Tentolouris N. Diabetic foot infections: An update in diagnosis and management. Curr Diab Rep 2017; 17: 3. https://doi.org/10.1007/s11892-017-0831-1
45. Ramamoorthy V. Physiatrist's assessment and management of diabetic foot. Int J Health Sci Res. 2019; 9(9):219–223.
46. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, et al. Risk factors for foot infections in individuals with diabetes. Diabetes Care 2006; 29:1288–1293.
47. Peters EJ, Lavery LA, Armstrong DG. Diabetic lower extremity infection: Influence of physical, psychological, and social factors. J Diabetes complications 2005; 19:107–112.
48. Lavery LA, Peters EJ, Armstrong DG, Wendel CS, et al. Risk factors for developing osteomyelitis in patients with diabetic foot wounds. Diabetes Res Clin Pract 2009; 83:347–352.
49. Del Core MA, Ahn J, Lewis RB, Raspovic K.M, et al. The evaluation and treatment of diabetic foot ulcers and diabetic foot infections. Foot & Ankle Orthopaedics 2018; https://doi.org/10.1177/2473011418788864
50. Sen P, Demirdal T, Emir B. Meta-analysis of risk factors for amputation in diabetic foot infections. Diabetes/metabolism research and reviews 2019; 35(7): e3165. https://doi.org/10.1002/dmrr.3165
51. Gomes A, Teixeira C, Ferraz R, Prudêncio C, et al. Wound-healing peptides for treatment of chronic diabetic foot ulcers and other infected skin injuries. Molecules 2017; 22: 1743. https://doi.org/10.3390/molecules22101743
52. Cervantes-García E, Salazar-Schettino PM. Clinical and surgical characteristics of infected diabetic foot ulcers in a tertiary hospital of Mexico. Diabetic Foot & Ankle 2017; 8(1): 1367210. https://doi.org/10.1080/2000625X.2017.1367210 T
53. Lázaro-Martínez JL, García Álvarez Y, Tardáguila-García A, García Morales E. Optimal management of diabetic foot osteomyelitis: challenges and solutions. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2019;12 947–12959.
54. Lázaro-Martínez JL, Tardáguila-García A, García-Klepzig JL. Diagnostic and therapeutic update on diabetic foot osteomyelitis. Endocrinología, Diabetes y Nutrición (English ed) 2017; 64: 100–108.
55. Meyr AJ, Seo K, Khurana JS, Choksi R, et al. Level of agreement with a multi-test approach to the diagnosis of diabetic foot osteomyelitis. J Foot Ankle Surg 2018; 57:1137–9.
56. Aragón-Sánchez J, Lipsky BA. Modern management of diabetic foot osteomyelitis. The when, how and why of conservative approaches. Expert Review of Anti-infective Therapy2018; 16 (1): 35–50. https://doi.org/10.1080/14787210.2018.1417037
57. Senneville E. Editorial Commentary: Probe-to-bone test for detecting diabetic foot osteomyelitis: rapid, safe, and accurate-but for which patients? Clin Infect Dis 2016; 63: 949–50.
58. Lam K, van Asten SA, Nguyen T, La Fontaine J, et al. Diagnostic accuracy of probe to bone to detect osteomyelitis in the diabetic foot: A systematic review. Clin Infect Dis 2016; 63:944–948.
59. Elamurugan TP, Jagdish S, Kate V, Parija SC. Role of bone biopsy specimen culture in the management of diabetic foot osteomyelitis. International Journal of Surgery 2011; 9(3): 214–216. https://doi.org/10.1016/j.ijsu.2010.11.011
60. Hobizal K.B, Wukich D.K. Diabetic foot infections: current concept review. Diabetic Foot & Ankle 2012; 3(1); 18409. https://doi.org/10.3402/dfa.v3i0.18409
61. Ong E, Farran S, Salloum M. Does everything that's counted count? Value of inflammatory markers for following therapy and predicting outcome in diabetic foot infection. The International Journal of Lower Extremity Wounds 2017; 16(2). https://doi.org/10.1177/1534734617700539
62. Uzun G, Solmazgul E, Curuksulu H, et al. Procalcitonin as a diagnostic aid in diabetic foot infections. Tohoku J Exp Med 2007; 213:305–312.

63. Park JH, Suh DH, Kim HJ, Lee YI, et al. Role of procalcitonin in infected diabetic foot ulcer. Diabetes Res Clin Pract 2017; 128:51–57.

64. Singer AJ, Tassiopoulos A, Kirsner RS. Evaluation and management of lower-extremity ulcers. N Engl J Med 2017; 377:1559–1567. https://doi.org/10.1056/NEJMra1615243

65. Meloni M, Izzo V, Giurato L, Brocco E, et al. Procalcitonin is a prognostic marker of hospital outcomes in patients with critical limb ischemia and diabetic foot infection. Journal of Diabetes Research 2019, Article ID 4312737, 5. https://doi.org/10.1155/2019/4312737

66. Umapathy D, Dornadula S, Rajagopalan A, et al. Potential of circulatory procalcitonin as a biomarker reflecting inflammation among South Indian diabetic foot ulcers. J Vasc Surg 2018; 67:1283–1291 e2.

67. Ozer Balin S, Sagmak Tartar A, Ugur K, et al. Pentraxin-3: A new parameter in predicting the severity of diabetic foot infection? Int Wound J 2019; 16(3): 659–664. https://doi.org/10.1111/iwj.13075

68. Ramanujam CL, Han D, Zgonis T. Medical imaging and laboratory analysis of diagnostic accuracy in 107 consecutive hospitalized patients with diabetic foot osteomyelitis and partial foot amputations. Foot Ankle Spec 2018; 11:433–443.

69. Mendes JJ, Marques-Costa A, Vilela C, Neves J, et al. Clinical and bacteriological survey of diabetic foot infections in Lisbon. Diabetes Research and Clinical Practice 2012; 95(1):153–161. https://doi.org/10.1016/j.diabres.2011.10.001

70. Slater R, Lazarovitch T, Boldur I, Ramot Y, et al. Swab cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. Diabetic medicine 2004; 21(7):705–709.

71. Dinh MT, Abad CL, Safdar N. Diagnostic accuracy of the physical examination and imaging tests for osteomyelitis underlying diabetic foot ulcers: Meta-analysis. Clin Infect Dis 2008; 47:519–527.

72. Cildag MB, Ertugrul BM, Koseoglu OF, Cildag S, et al. Angiographic assessment of atherosclerotic load at the lower extremity in patients with diabetic foot and charcot neuroarthropathy. J Chin Med Assoc 2018; 81:56570.

73. Lauri C, Tamminga M, Glaudemans AWJM, et al. Detection of osteomyelitis in the diabetic foot by imaging techniques: A systematic review and meta-analysis comparing MRI, white blood cell scintigraphy, and FDG-PET. Diabetes Care 2017; 40:1111–1120.

74. Lipsky BA, Berendt AR, Embil J, de Lalla F. Diagnosing and treating diabetic foot infections. Diabetes/Metabolism Research and Reviews. 2004; 20(S1): S56-S64. https://doi.org/10.1002/dmrr.441

75. Jeffcoate WJ, Lipsky BA. Controversies in diagnosing and managing osteomyelitis of the foot in diabetes. Clinical Infectious Diseases. 2004; 39(2):S115-S122 https://doi.org/10.1086/383272

76. Gariani K, Lebowitz D, von Dach E, Kressmann B, et al. Remission in diabetic foot infections: Duration of antibiotic therapy and other possible associated factors. Diabetes Obes Metab 2019; 21:244–251. https://doi.org/10.1111/dom.13507

77. Vouillarmet J, Morelec I, Thivolet C. Assessing diabetic foot osteomyelitis remission with white blood cell SPECT/CT imaging. Diabet Med 2014; 31:1093–1099. https://doi.org/10.1111/dme.12445

78. Senneville E, Melliez H, Beltrand E, et al. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clin Infect Dis 2006; 42:57–62. https://doi.org/10.1086/498112

79. Senneville E, Morant H, Descamps D, et al. Needle puncture and transcutaneous bone biopsy cultures are inconsistent in patients with diabetes and suspected osteomyelitis of the foot. Clin Infect Dis 2009; 48:888–893. https://doi.org/10.1086/597263

80. Abbas ZG, Lutale JK, Ilondo MM, Archibald LK. The utility of Gram stains and culture in the management of limb ulcers in persons with diabetes. Int Wound J 2012; 9:677–682. https://doi.org/10.1111/j.1742-481X.2011.00937.x

81. Johani K, Fritz BG, Bjarnsholt T, Lipsky BA, et al. Understanding the microbiome of diabetic foot osteomyelitis: insights from molecular and microscopic approaches. Clin Microbiol Infect 2019; 25(3): 332–339. https://doi.org/10.1016/j.cmi.2018.04.036

82. Sella EJ, Grosser DM. Imaging modalities of the diabetic foot. Clinics in podiatric medicine and surgery. 2003; 20(4):729–740. https://doi.org/10.1016/S0891-8422(03)00070-3

83. Lin SY, Lin NY, Huang YY, Hsieh CC, et al. Methicillin-resistant Staphylococcus aureus nasal carriage and infection among patients with diabetic foot ulcer. J of Microbiology, immunology and infection 2020; 53: 292–299. https://doi.org/10.1016/j.jmii.2018.03.005

84. Mergenhagen KA, Croix M, Starr KE, Sellick JA, et al. Utility of methicillin-resistant Staphylococcus Aureus nares screening for patients with a diabetic foot infection. Antimicrob Agents Chemother 2020; 64:e02213–19. https://doi.org/10.1128/AAC.02213-19.

85. Selva Olid A, Sola I, Barajas-Nava LA, Gianneo OD, et al. Systemic antibiotics for treating diabetic foot infections. Cochrane Database Syst Rev 2015:CD009061.

86. Lipsky BA, Dryden M, Gottrup F, Nathwani D, et al. Antimicrobial stewardship in wound care: A Position Paper from the British Society for Antimicrobial Chemotherapy and European Wound Management Association. J Antimicrob Chemother 2016; 71(11):3026–3035. https://doi.org/10.1093/jac/dkw287

87. Uckay I, Berli M, Sendi P, Lipsky BA. Principles and practice of antibiotic stewardship in the management of diabetic foot infections. Curr Opin Infect Dis 2019; 32 (2):95–101. https://doi.org/10.1097/QCO.0000000000000530

88. Zgonis T, Roukis TS. A systematic approach to diabetic foot infections. Adv in Therapy 2005; 22, 244. https://doi.org/10.1007/BF02849934

89. Siami G, Christou N, Eiseman I, Tack KJ. Clinafloxacin versus piperacillin-tazobactam in treatment of patients with severe skin and soft tissue infections. Antimicrob Agents Chemother 2001; 45:525–531. https://doi.org/10.1128/AAC.45.2.525-531.2001

90. Percival SL, Malone M, Mayer D, Salisbury AM, et al. Role of anaerobes in polymicrobial communities and biofilms complicating diabetic foot ulcers. Int Wound J 2018; 15:776–782.https://doi.org/10.1111/iwj.12926

91. Charles PG, Uckay I, Kressmann B, Emonet S, et al. The role of anaerobes in diabetic foot infections. Anaerobe 2015; 34:8–13. https://doi.org/10.1016/j.anaerobe.2015.03.009

92. Lipsky BA, Itani K, Norden C, Group LDFIS. Treating foot infections in diabetic patients: a randomized, multicenter, open-label trial of linezolid versus ampicillin-sulbactam/amoxicillin-clavulanate. Clinical Infectious Diseases. 2004; 38(1):17–24. https://doi.org/10.1086/380449

93. Wakefield MC, Kan VL, Arora S, Weiswasser JM, et al. Nonoperative management of diabetic foot infections. In: Diabetic foot: Lower extremity arterial disease and limb salvage. Philadelphia: Lippincott Williams & Wilkins; 2006 Chapter 6, pages: 43–48.

94. Uckay I, Jornayvaz FR, Lebowitz D, Gastaldi G, et al. An overview on diabetic foot infections, including issues related to associated pain, hyperglycemia and limb ischemia. Curr Pharm Des 2018; 24(12):1243–1254. https://doi.org/10.2174/1381612824666180302145754

95. Commons RJ, Raby E, Athan E, et al. Managing diabetic foot infections: a survey of Australasian infectious diseases clinicians. J Foot Ankle Res 2018; 11:13. https://doi.org/10.1186/s13047-018-0256-3

96. Sumpio BE. Contemporary evaluation and management of the diabetic foot. Scientifica 2012; Article ID 435487. https://doi.org/10.6064/2012/435487

97. Lesens O, Desbiez F, Theis C, et al. Staphylococcus aureus-related diabetic osteomyelitis: medical or surgical management? A French and Spanish retrospective cohort. Int J Low Extrem Wounds 2015; 14:284–290. https://doi.org/10.1177/1534734614559931

98. Morrison WB, Ledermann HP. Work-up of the diabetic foot. Radiologic Clinics 2002; 40 (5):1171–1192. https://doi.org/10.1016/S0033-8389(02)00036-2

99. Ruke MG, Savai J. Diabetic foot infection, biofilm & new management strategy. Diab Res Open Access 2019; 1(1):7–22. https://doi.org/10.36502/2019/droa.6152

100. van Schie CL, Vermigli C, Carrington AL, Boulton A. Muscle weakness and foot deformities in diabetes: Relationship to neuropathy and foot ulceration in Caucasian diabetic men. Diabetes Care 2004; 27(7):1668–1673. https://doi.org/10.2337/diacare.27.7.1668

101. Shively VP, Feinglass J, Martin GJ, Huang ME, et al. How 'preventable' are lower extremity amputations? A qualitative study of patient perceptions of precipitating factors. Disability and Rehabilitation J 2012; 34(25): 2158–2165. https://doi.org/10.3109/09638288.2012.677936

102. Game F, Jeffcoate W. The Charcot foot: Neuropathic osteoarthropathy. Adv Skin Wound Care 2013; 26 (9):421–428. https://doi.org/10.1097/01.ASW.0000433789.25992.e5

103. Petrova N, Dew T, Musto R, Sherwood R, et al. Inflammatory and bone turnover markers in a cross-sectional and prospective study of acute Charcot osteoarthropathy. Diabetic Medicine 2015; 32(2):267–273. https://doi.org/10.1111/dme.12590

104. Kaynak G, Birsel O, Güven MF, Oğüt T. An overview of the Charcot foot pathophysiology. Diabet Foot Ankle 2013; Article: 21117. https://doi.org/10.3402/dfa.v4i0.21117

105. Salini D, Harish K, Minnie P, et al. Prevalence of Charcot arthropathy in type 2 diabetes patients aged over 50 years with severe peripheral neuropathy: A retrospective study in a tertiary care south Indian hospital. Indian J Endocrinol Metab 2018; 22(1):107–111. https://doi.org/10.4103/ijem.IJEM_257_17

106. Rogers LC, Frykberg RG, Armstrong DG, et al. The Charcot foot in diabetes. J Am Podiatr Med Assoc 2011; 101 (5): 437–446.https://doi.org/10.7547/1010437

107. Botek G, Anderson MA, Taylor R. Charcot neuro arthropathy: An often overlooked complication of diabetes. Cleve Clin J Med 2010; 77(9):593–599. doi:https://doi.org/10.3949/ccjm.77a.09163

108. Bem R, Jirkovská A, Dubsky M, et al. Role of quantitative bone scanning in the assessment of bone turnover in patients with Charcot foot. Diabetes Care 2010; 33:348.

109. Ferreira RC, Gonçalez DH, Filho JM, et al. Midfoot charcot arthropathy in diabetic patients: Complication of an epidemic disease. Revista Brasileira de Ortopedia (English Edition) Rev Bras Ortop 2012; 47(5):616–625. https://doi.org/10.1016/S2255-4971(15)30013-6

110. Hartemann-Heurtier A, Van GH, Grimaldi A. The Charcot foot. Lancet 2002; 360 (9347): 1776–1779. DOI:https://doi.org/10.1016/S0140-6736(02)11671-0

111. Dalla Paola L, Faglia E. Treatment of diabetic foot ulcer: An overview strategies for clinical approach. Curr Diabetes Rev 2006; 2 (4): 431–447.

112. Ong E, Farran S, Salloum M, et al. The role of inflammatory markers: WBC, CRP, ESR, and neutrophil-to-lymphocyte ratio (NLR) in the diagnosis and management of diabetic foot infections. Open Forum Infectious Diseases 2015; 2 (s1):1526. https://doi.org/10.1093/ofid/ofv133.1079

113. Prompers L, Huijberts M, Apelqvist J, Jude E, et al. High prevalence of ischemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. Diabetologia 2007; 50(1):18–25. https://doi.org/10.1007/s00125-006-0491-1.

114. Morbach S, Furchert H, Groeblinghoff U, Hoffmeier H, et al. Long-term prognosis of diabetic foot patients and their limbs. Dia Care. 2012; 35(10):2021–2027. https://doi.org/10.2337/dc12-0200.

115. Rigato M, Pizzol D, Tiago A, Putoto G, et al. Characteristics, prevalence, and outcomes of diabetic foot ulcers in Africa. A systemic review and meta-analysis. Diabetes Research and Clinical Practice 2018; 142:63–73. doi:https://doi.org/10.1016/j.diabres.2018.05.016.

116. Younis BB, Shahid A, Arshad R, Khurshid S, et al. Frequency of foot ulcers in people with type 2 diabetes, presenting to specialist diabetes clinic at a Tertiary Care Hospital, Lahore, Pakistan. BMC Endocr Disord 2018; 18(1):53. https://doi.org/10.1186/s12902-018-0282-y.

117. Junrungsee S, Kosachunhanun N, Wongthanee A, Rerkasem K. History of foot ulcers increases mortality among patients with diabetes in Northern Thailand. Diabet Med 2011; 28(5):608–611. doi:https://doi.org/10.1111/j.14645491.2011.03262.x.

118. Spreen MI, Gremmels H, Teraa M, Sprengers RW, et al. Diabetes is associated with decreased limb survival in patients with critical limb ischemia: pooled data from two randomized controlled trials. Dia Care. 2016; 39(11):2058–2064. https://doi.org/10.2337/dc16-0850.
119. Elgzyri T, Larsson J, Thörne J, Eriksson K-F, et al. Outcome of ischemic foot ulcer in diabetic patients who had no invasive vascular intervention. Eur J Vasc Endovasc Surg 2013; 46(1):110–117. doi:https://doi.org/10.1016/j.ejvs.2013.04.013.
120. Hart T, Milner R, Cifu A. Management of a diabetic foot. JAMA 2017; 318(14):1387–1388. doi:https://doi.org/10.1001/jama.2017.11700.
121. Diabetic foot problems Prevention and management, NICE Clinical Guideline19 (Methods, evidence and recommendations), Published August 2015, updated May 2016. Commissioned by the National Institute for Health and Care Excellence.4.6.6.P:96.
122. Albert N, Bozkurt B, Brindis FRG, Curtis LH, et al. Management of patients with peripheral artery disease (Compilation of 2005 and 2011 ACCF/AHA Guideline Recommendations). Journal of the American College of Cardiology 2013; 61(14): 1555–1570. https://doi.org/10.1016/j.jacc.2013.01.004
123. Wukich DK, Shen W, Raspovic KM, Suder NC, et al. Noninvasive arterial testing in patients with diabetes: A guide for foot and ankle surgeons. Foot Ankle Int 2015; 36(12):1391–1399. https://doi.org/10.1177/1071100715593888.
124. Bunte MC, Jacob J, Nudelman B, Shishehbor MH. Validation of the relationship between ankle-brachial and toe brachial indices and infra genicular arterial patency in critical limb ischemia. Vasc Med 2015; 20(1):23–29. https://doi.org/10.1177/1358863X14565372.
125. Wang Z, Hasan R, Firwana B, Elraiyah T, et al. A systematic review and meta-analysis of tests to predict wound healing in diabetic foot. YMVA 2016; 63(2):29S–U99. doi:https://doi.org/10.1016/j.jvs.2015.10.004.
126. Schreuder SM, Nieuwdorp M, Koelemay MJW, Bipat S, et al. Testing the sympathetic nervous system of the foot has a high predictive value for early amputation in patients with diabetes with a neuroischemic ulcer. BMJ Open Diabetes Res Care 2018; 6(1):e000592. https://doi.org/10.1136/bmjdrc-2018-000592.
127. Ruangsetakit C, Chinsakchai K, Mahawongkajit P, Wongwanit C, et al. Transcutaneous oxygen tension: a useful predictor of ulcer healing in critical limb ischemia. Journal of Wound Care 2013; 19 (5). https://doi.org/10.12968/jowc.2010.19.5.48048
128. Gazzaruso C, Coppola A, Falcone C, Luppi C, et al. Transcutaneous oxygen tension as a potential predictor of cardiovascular events in Type 2 diabetes. Comparison with ankle-brachial index. Diabetes Care 2013; 36(6): 1720–1725. https://doi.org/10.2337/dc12-1401
129. Prompers L, Schaper N, Apelqvist J, Edmonds M, et al. Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral artery disease. The EURODIALE Study. Diabetologia 2008; 51(5):747–755. https://doi.org/10.1007/s00125008-0940-0.
130. Fisher TK, Scimeca CL, Bharara M, Mills JLS, et al. A stepwise approach for surgical management of diabetic foot infections. Journal of the American Podiatric Medical Association 2010; 100(5):401–405. https://doi.org/10.7547/1000401.
131. Schaper NC, Van Netten JJ, Apelqvist J, Bus SA, Hinchliffe RJ, et al. IWGDF Practical Guidelines on the prevention and management of diabetic foot disease. Diab Metab Res Rev 2016; 32(51):7–15. https://doi.org/10.1002/dmrr.2695
132. Lipsky BA, Senneville E, Abbas ZG, Aragón-Sánchez J, et al. IWGDF guideline on the diagnosis and treatment of foot infection in people with diabetes. Diab Metab Res Rev 2020; 36(s1). https://doi.org/10.1002/dmrr.3280
133. Tallis A, Motley TA, Wunderlich RP, Dickerson JE, et al. Clinical and economic assessment of diabetic foot ulcer debridement with collagenase: Results of a randomized controlled study. Clin Ther 2013; 35: 1805–1820 . https://doi.org/10.1016/j.clinthera.2013.09.013.
134. Lebrun E, Tomic-Canic M, Kirsner RS. The role of surgical debridement in healing of diabetic foot ulcers. Wound Repair Regen 2010; 18: 433–438. https://doi.org/10.1111/j.1524-475X.2010.00619.x.

135. Edwards J, Stapley S. Debridement of diabetic foot ulcers. Cochrane Database Syst Rev 2010; (1): CD003556. https://doi.org/10.1002/14651858.CD003556.

136. Brem H, Sheehan P, Boulton AJ. Protocol for treatment of diabetic foot ulcers. Am J Surg 2004; 187: 1S–10S. https://doi.org/10.1016/S0002-9610(03)00299-X.

137. DiPreta JA. Outpatient assessment and management of the diabetic foot. Med Clin North Am 2014; 98: 353–373. https://doi.org/10.1016/j.mcna.2013.10.010.

138. Rayman G, Vas P, Dhatariya K, Driver V, et al. Guidelines on use of interventions to enhance healing of chronic foot ulcers in diabetes (IWGDF 2019 update). Diabetes Metab Res Rev. 2020; 36(S1):e3283. https://doi.org/10.1002/dmrr.3283.

139. Cardinal M, Eisenbud DE, Armstrong DG, Zelen C, et al. Serial surgical debridement: A retrospective study on clinical outcomes in chronic lower extremity wounds. Wound Repair Regen 2009; 17: 306–311. https://doi.org/10.1111/j.1524475X.2009.00485.x.

140. Jain AC. A new classification (grading system) of debridement in diabetic lower limbs-an improvisation and standardization in practice of diabetic lower limb salvage around the world. Medicine Science 2014; 3: 991–1001. https://doi.org/10.5455/medscience.2013.02.8093.

141. Enoch S, Harding K. Wound bed preparation: the science behind the removal of barrier to healing. Wounds 2003; 15: 213–229.

142. Sherman RA. Maggot therapy for foot and leg wounds. Int J Low Extrem Wounds 2002; 1: 135–142. https://doi.org/10.1177/1534734602001002009.

143. Sherman RA. Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy. Diabetes Care 2003; 26: 446–451. https://doi.org/10.2337/diacare.26.2.446.

144. van Veen LJ. Maggot debridement therapy: A case study. J Wound Ostomy Continence Nurs 2008; 35: 432–436. https://doi.org/10.1097/01.WON.0000326667.62884.51.

145. Armstrong DG, Salas P, Short B, Martin BR, et al. Maggot therapy in "lower-extremity hospice" wound care: Fewer amputations and more antibiotic-free days. J Am Podiatr Med Assoc 2005; 95: 254–257. PMID: 15901812.

146. Paul AG, Ahmad NW, Lee HL, Ariff AM, et al. Maggot debridement therapy with Lucilia cuprina: A comparison with conventional debridement in diabetic foot ulcers. Int Wound J 2009; 6: 39–46. https://doi.org/10.1111/j.1742-481X.2008.00564.x.

147. Scott RG, Loehne HB. 5 questions – and answers – about pulsed lavage. Adv Skin Wound Care 2000; 13: 133–134. PMID: 11075009.

148. Armstrong DG, Nguyen HC, Lavery LA, van Schie CH, et al. Off-loading the diabetic foot wound: a randomized clinical trial. Diabetes Care 2001; 24: 1019–1022. https://doi.org/10.2337/diacare.24.6.1019.

149. Armstrong DG, Lavery LA, Nixon BP, Boulton AJ. It's not what you put on, but what you take off: Techniques for debriding and off-loading the diabetic foot wound. Clin Infect Dis 2004; 39 (S2): S92-S99. https://doi.org/10.1086/383269.

150. Rathur HM, Boulton AJ. The diabetic foot. Clin Dermatol 2007; 25: 109–120. https://doi.org/10.1016/j.clindermatol.2006.09.015.

151. Wukich DK, Motko J. Safety of total contact casting in high-risk patients with neuropathic foot ulcers. Foot & Ankle International J 2004; 25 (8): 556–560. https://doi.org/10.1177/107110070402500808

152. Boulton AJ. Pressure and the diabetic foot: clinical science and offloading techniques. Am J Surg 2004; 187: 17S–24S. https://doi.org/10.1016/S0002-9610(03)00297-6.

153. Cavanagh PR, Bus SA. Off-loading the diabetic foot for ulcer prevention and healing. J Vasc Surg 2010; 52: 37S–43S. https://doi.org/10.1016/j.jvs.2010.06.007.

154. Frykberg RG. Diabetic foot ulcers: Pathogenesis and management. Am Fam Physician 2002; 66: 1655–1662.

155. Collings R, Freeman J, M. Latour, Paton J. Footwear and insole design features for offloading the diabetic at risk foot—A systematic review and meta-analyses. Endocrinol Diab Metab 2020; 00:e00132. https://doi.org/10.1002/edm2.132.

156. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA 2005; 293: 217–228. doi:https://doi.org/10.1001/jama.293.2.217

157. Moura LI, Dias AM, Carvalho E, de Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment – A review. Acta Biomater 2013; 9:7093–7114. https://doi.org/10.1016/j.actbio.2013.03.033.

158. Fard AS, Esmaelzadeh M, Larijani B. Assessment and treatment of diabetic foot ulcer. Int J Clin Pract 2007; 61: 1931–1938. https://doi.org/10.1111/j.1742-1241.2007.01534.x.

159. Hilton JR, Williams DT, Beuker B, Miller DR, et al. Wound dressings in diabetic foot disease. Clin Infect Dis 2004; 39 (S2): S100-S103. https://doi.org/10.1086/383270.

160. Oliveira N, Rosa P, Borges L, Dias E, et al. Treatment of diabetic foot complications with hyperbaric oxygen therapy: a retrospective experience. Foot Ankle Surg 2014; 20: 140–143. https://doi.org/10.1016/j.fas.2014.02.004.

161. Landau Z, Sommer A, Miller EB. Topical hyperbaric oxygen and low-energy laser for the treatment of chronic ulcers. European Journal of Internal Medicine 2006; 17 (4): 272–275. https://doi.org/10.1016/j.ejim.2005.11.028

162. Thackham JA, McElwain DL, Long RJ. The use of hyperbaric oxygen therapy to treat chronic wounds: A review. Wound Repair Regen 2008; 16: 321–330. doi:https://doi.org/10.1111/j.1524-475X.2008.00372.x

163. Gill AL, Bell CN. Hyperbaric oxygen: Its uses, mechanisms of action and outcomes. QJM 2004; 97: 385–395. https://doi.org/10.1093/qjmed/hch074.

164. Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. World J Diabetes 2015; 6(1): 37–53. https://doi.org/10.4239/wjd.v6.i1.37.

165. Thom SR. Hyperbaric oxygen: Its mechanisms and efficacy. Plast Reconstr Surg 2011; 127 (S1): 131S–141S. https://doi.org/10.1097/PRS.0b013e3181fbe2bf.

166. Aalaa M, Malazy OT, Sanjari M, Peimani M, et al. Nurses' role in diabetic foot prevention and care; a review. J Diabetes Metab Disord 2012; 11: 24. https://doi.org/10.1186/2251-6581-11-24

167. Armstrong DG, Lavery LA. Negative pressure wound therapy after partial diabetic foot amputation: A multicentre, randomized controlled trial. Lancet 2005; 366: 1704–1710. https://doi.org/10.1016/S0140-6736(05)67695-7

168. Vikatmaa P, Juutilainen V, Kuukasjärvi P, Malmivaara A. Negative pressure wound therapy: A systematic review on effectiveness and safety. Eur J Vasc Endovasc Surg 2008; 36:438–448. https://doi.org/10.1016/j.ejvs.2008.06.010

169. DeFranzo AJ, Argenta LC, Marks MW, Molnar JA, et al. The use of vacuum-assisted closure therapy for the treatment of lower-extremity wounds with exposed bone. Plast Reconstr Surg 2001; 108:1184–1191 https://doi.org/10.1016/j.cpm.2007.03.011.

170. Espensen EH, Nixon BP, Lavery LA, Armstrong DG. Use of subatmospheric (VAC) therapy to improve bioengineered tissue grafting in diabetic foot wounds. J Am Podiatr Med Assoc 2002; 92: 395–397. https://doi.org/10.7547/87507315-92-7-395

171. Venturi ML, Attinger CE, Mesbahi AN, Hess CL, et al. Mechanisms and clinical applications of the vacuum-assisted closure (VAC) Device: A review. Am J Clin Dermatol 2005; 6:185–194. PMID: 15943495.

172. Kim PJ, Heilala M, Steinberg JS, Weinraub GM. Bioengineered alternative tissues and hyperbaric oxygen in lower extremity wound healing. Clin Podiatr Med Surg 2007; 24(3):529–546. https://doi.org/10.1016/j.cpm.2007.03.011

173. Richmond NA, Vivas AC, Kirsner RS. Topical and biologic therapies for diabetic foot ulcers. Med Clin North Am 2013; 97:883–898. https://doi.org/10.1016/j.mcna.2013.03.014

174. Futrega K, King M, Lott WB, Doran MR. Treating the whole not the hole: Necessary coupling of technologies for diabetic foot ulcer treatment. Trends Mol Med 2014; 20: 137–142. https://doi.org/10.1016/j.molmed.2013.12.004.

175. Kirsner RS, Warriner R, Michela M, Stasik L, et al. Advanced biological therapies for diabetic foot ulcers. Arch Dermatol 2010; 146: 857–862. https://doi.org/10.1001/archdermatol.2010.164

176. Dinh TL, Veves A. The efficacy of Apligraf in the treatment of diabetic foot ulcers. Plast Reconstr Surg 2006; 117:152S–157S. https://doi.org/10.1097/01.prs.0000222534.79915.d3

177. Ramundo J, Gray M. Enzymatic wound debridement. J Wound Ostomy Continence Nurs 2008; 35: 273–280. doi:https://doi.org/10.1097/01.WON.0000319125.21854.78

178. Hirase T, Ruf E, Surani S, Ratnani I. Topical application of platelet-rich plasma for diabetic foot ulcers: A systematic review. World J Diabetes 2018; 9(10): 172–179. 2018 October 15 https://doi.org/10.4239/wjd.v9.i10.172

179. Dai J, Jiang C, Sun Y, Chen H. Autologous platelet-rich plasma treatment for patients with diabetic foot ulcers: A meta-analysis of randomized studies. Journal of Diabetes and Its Complications. doi:https://doi.org/10.1016/j.jdiacomp.2020.107611. In Press.

180. Elsaid A, El-Said M, Emile S, Youssef M, et al. Randomized controlled trial on autologous platelet-rich plasma versus saline dressing in treatment of non-healing diabetic foot ulcers. World J Surg 2020; 44:1294–1301. https://doi.org/10.1007/s00268-019-05316-0

181. Seaman S. The role of the nurse specialist in the care of patients with diabetic foot ulcers. Foot Ankle Int 2005; 26:19–26. https://doi.org/10.1177/107110070502600104.

182. Barnes R, Shahin Y, Gohil R, Chetter I. Electrical stimulation vs. standard care for chronic ulcer healing: a systematic review and meta-analysis of randomized controlled trials. Eur J Clin Invest 2014; 44: 429–440. https://doi.org/10.1016/j.ejvs.2008.06.010.

183. Nather A, Bee CS, Huak CY, Chew JL, et al. Epidemiology of diabetic foot problems and predictive factors for limb loss. J Diabetes Complications 2008; 22: 77–82. https://doi.org/10.1016/j.jdiacomp.2007.04.004.

184. Beckmann K, Meyer-Hamme G, Schröder S. Low level laser therapy for the treatment of diabetic foot ulcers: A critical survey. Evidence-Based Complementary and Alternative Medicine .2014, Article ID 626127, 9 pages. https://doi.org/10.1155/2014/626127.

185. Landaua Z, Schattnerb A. Topical hyperbaric oxygen and low energy laser therapy for chronic diabetic foot ulcers resistant to conventional treatment Yale J Biol Med 2001; 74(2): 95–100. PMID: 11393266

186. Izadi M, Jonaidi Jafari N, Sadat Hosseini M, Saafaat O. Therapeutic effects of ozone in patients with diabetic foot ulcers: review of the literature, review article. Biomedical Research 2017; 28(18). Available on: https://www.biomedres.info/biomedical-research/therapeutic-effects-of-ozone-in-patients-with-diabetic-foot-ulcers-review-of-the-literature-8469.html

187. Kushmakov R, Gandhi J, Seyam O, Jiang W. Ozone therapy for diabetic foot. Med Gas Res 2018; 8(3):111–115. https://doi.org/10.4103/2045-9912.241076

188. Izadi M, Kheirjou R, Mohammad Pour R, Aliyoldashi M.H, et al. Efficacy of comprehensive ozone therapy in diabetic foot ulcer healing. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2019; 13:822–825. doi:https://doi.org/10.1016/j.dsx.2018.11.060

189. Borrelli E, De Monte A, Bocci V. Oxygen ozone therapy in the integrated treatment of chronic ulcer: A case series report. International Journal of Recent Scientific Research 2015; 6(5):4132e6.

190. Rezaeinezhad A, Eslami P, Mirmiranpour H & Ghomi H. The effect of cold atmospheric plasma on diabetes-induced enzyme glycation, oxidative stress, and inflammation; in vitro and in vivo. Scientific RepoRtS 2019; 9:19958. https://doi.org/10.1038/s41598-019-56459-y

191. Kisch T, Helmke A, Schleusser S, Song J. Improvement of cutaneous microcirculation by cold atmospheric plasma (CAP): Results of a controlled, prospective cohort study. Micro vascular Research 2016; 104: 55–62. https://doi.org/10.1016/j.mvr.2015.12.002

192. He R, Li Q, Yu M, Wang T, et al. The efficacy and safety of cold atmospheric plasma as a novel therapy for diabetic wound in vivo and in vitro. Diabetes 2019; 68(1). https://doi.org/10.2337/db19-646-P

193. Wang J, Zeng X.X, Cai W, Han ZB, et al. Safety and efficacy of placenta-derived mesenchymal stem cell treatment for diabetic patients with critical limb ischemia: A pilot study. Exp Clin Endocrinol Diabetes 2020. https://doi.org/10.1055/a-0978-4972

194. Ahmadi Ashtiani H, Firooz A, Rastegar H, Askaripour A. Healing potential of stem cells for diabetic ulcers. jdc 2020; 10 (4):252–270. Available on: http://jdc.tums.ac.ir/article-1-5419-en.html

195. Adam KM, Mahmoud SM, Mahadi SI,Widatalla AH, et al. Extended leg infection of diabetic foot ulcers: Risk factors and outcome. Journal of Wound Care 2011; 20(9):440–444. https://doi.org/10.12968/jowc.2011.20.9.440

196. Baker SR, Stacey MC, Singh G, Hoskin SE, et al. Aetiology of chronic leg ulcers. Eur J Vasc Surg 1992; 6: 245–251.

197. Wallaer JB, Nolan BW, Adams J, Stanley AC, et al. The impact of diabetes on postoperative outcomes following lower-extremity bypass surgery. Journal of Vascular Surgery 2012; 56(5):1317–1323. https://doi.org/10.1016/j.jvs.2012.04.011

198. Han HS, Kang SB. Relations between long-term glycemic control and postoperative wound and infectious complications after total knee arthroplasty in Type 2 diabetics. Clin Orthop Surg 2013; 5(2):118–123. https://doi.org/10.4055/cios.2013.5.2.118

199. Tsang STG, Gaston P. Adverse peri-operative outcomes following elective total hip replacement in diabetes mellitus. A systematic review and meta-analysis of cohort studies. Bone Joint J 2013; 95-B: 1474–9. doi:https://doi.org/10.1302/0301-620X.95B11.3.

200. Kermers HM, Lewallen LW, Mabry TM, Berry DJ, et al. Diabetes mellitus, hyperglycemia, hemoglobin A1C and the risk of prosthetic joint infections in total hip and knee arthroplasty. The Journal of Arthroplasty 2015; 30 (3):439–443. https://doi.org/10.1016/j.arth.2014.10.009

201. Pedersen AB, Mehnert F, Johnsen SP, Sørensen HT. Risk of revision of a total hip replacement in patients with diabetes mellitus. A population-based follow up study. The Journal of Bone and Joint Surgery 2010; 92 (7): https://doi.org/10.1302/0301-620X.92B7.

202. Gohel MS, Taylor M, Earnshaw JJ, Heather BP, et al. Risk factors for delayed healing and recurrence of chronic venous leg ulcers—An analysis of 1324 legs. Eur J Vasc Endovasc Surg 2005; 29:74–77. doi:https://doi.org/10.1016/j.ejvs.2004.10.002

203. Allen KB, Heimansohn DA, Robison RJ, Schier JJ, et al. Risk factors for leg wound complications following endoscopic versus traditional saphenous vein harvesting. The Heart Surgery Forum 2000; 3(4):325–330.

204. Papanas N, Papachristou S. COVID-19 and diabetic foot: Will the lamp burn bright?. The International Journal of Lower Extremity Wounds 2020; https://doi.org/10.1177/1534734620921382

205. Tao F, Tang X, Tao H, Luo Y, et al. Surgical treatment of diabetic foot ulcers during the COVID-19 pandemic in China. Journal of Diabetes and Its Complications 2020. https://doi.org/10.1016/j.jdiacomp.2020.107622

206. Rogers LC, Lavery LA, Joseph WS, Armstrong DG. All feet on deck—the role of podiatry during the covid-19 pandemic: preventing hospitalizations in an overburdened healthcare system, reducing amputation and death in people with diabetes. Journal of the American Podiatric Medical Association 2020. https://doi.org/10.7547/20-051

207. Armstrong DG, Giovinco N, Mills JL, Rogers LC. FaceTime for physicians: Using real time mobile phone-based videoconferencing to augment diagnosis and care in telemedicine. Eplasty 2011; 11:e23.

Chapter 7
Incidence, Complications, and Novel Treatment Strategies: Osteomyelitis

Check for updates

Catherine G. Ambrose, James F. Kellam, Lindsay Crawford, and Timothy S. Achor

Abstract Osteomyelitis, or infection of the bone, is a relatively rare musculoskeletal condition. However, it can be difficult to diagnose and treat, resulting in significant health-care expenditures and morbidity for the patient. Complications can be divided into two groups: general or systemic and specific. General complications are associated with the systemic effects of any disease process, while specific complications are a result of the disease process itself. This chapter describes the general complications associated with osteomyelitis such as systemic sepsis and chronic disease manifestations, as well as specific complications including osteonecrosis, multifocal osteomyelitis, malignant transformation in osteomyelitis, amputation, deformity, and fracture. Early diagnosis of pediatric osteomyelitis is key to urgent initiation of appropriate treatment, and this chapter also highlights recent innovations and emerging strategies that may be effective in treating osteomyelitis in both the pediatric and adult patient.

Keywords Osteomyelitis · Infection · Bone · Sepsis · Chronic · Acute · Pediatric · Adult · Novel treatment strategies

7.1 Introduction

Osteomyelitis, or infection of the bone, is a relatively rare musculoskeletal condition. However, it can be difficult to diagnose and treat, resulting in significant health-care expenditures and morbidity for the patient. The diagnosis of osteomyelitis can be challenging as up to 47% of confirmed osteomyelitis cases can be culture negative [1, 2]. Negative cultures can result from improper sample collection, improper microbiological testing, biofilm bacteria, or antibiotic use prior to sample collection. Osteomyelitis can be difficult to treat as most antibiotics given

C. G. Ambrose (✉) · J. F. Kellam · L. Crawford · T. S. Achor
Department of Orthopaedic Surgery, McGovern Medical School, The University of Texas
Health Science Center of Houston, Houston, TX, USA
e-mail: catherine.g.ambrose@uth.tmc.edu

parenterally do not penetrate the bone well, bacteria with a biofilm are resistant to antibiotics and immune defenses, and bacteria can invade mammalian cells. Further complicating the treatment of osteomyelitis is the increasing incidence of infections due to antibiotic-resistant strains.

Waldvogel proposed a classification system for osteomyelitis [3–5] based on etiology: infection that spreads through the blood or infection that spreads to the bone contiguously from a local contamination. The first type, osteomyelitis that results from hematogenous spread, usually occurs in prepubertal children or the elderly and usually affects the vertebral bodies or metaphyseal region of long bones. Osteomyelitis that results from contiguous spread can be further subdivided into cases where the host tissue is well vascularized (as in cases that arise from trauma or surgery in a healthy subject) versus tissue that suffers from vascular insufficiency, such as the case in diabetic foot ulcers. In all three cases, the infection can be further categorized as either acute or chronic. A more detailed classification system, the Cierny-Mader system [6], is based on anatomical, clinical, and radiologic features. This system, while more complicated than the one proposed by Waldvogel, is specific which allows for dictating treatment and dynamic which allows for changes in host status.

7.2   Incidence

The true incidence of osteomyelitis is difficult to assess since some cases are probably undiagnosed or misdiagnosed, and most published reports of musculoskeletal infections do not explicitly report osteomyelitis numbers (they only report superficial versus deep infection rates). As osteomyelitis is a subset of "deep infection," we can presume that the incidence is not higher than that reported for deep infection, but we may not know the true rate.

There are however a few studies which report overall incidence of osteomyelitis. One such study investigated osteomyelitis cases over a 41-year period (1969–2009) in a single county in Minnesota [7]. Using the total population for the county over that period of time, the overall osteomyelitis incidence was calculated to be 21.8 cases per 100,000 person-years. It was found that the annual incidence was higher for men than women regardless of the age group studied and the annual incidence increased over time (the incidence increased from 11.4 cases per 100,000 person-years in the first decade studied to 24.4 in the last). The average age of the patient with osteomyelitis increased over time, the proportion of infections caused by *Staphylococcus* species decreased over time, and the proportion of culture-negative cases increased over time. Overall, over half of the cases were caused by staphylococcal species (44% *S. aureus* and 17% *S. epidermis*) with another 16% of the cases found to be caused by *Streptococcus* species. Thirteen percent of the osteomyelitis cases due to hematogenous spread were polymicrobial, whereas the percentage of polymicrobial cases increased to 35% and 40% in the cases of contiguous-spread infections without and with diabetes.

A study by Rubin and coauthors [8] investigated the infections in 1995 New York City hospitals. Although they studied all infections and were specifically interested in those caused by *S. aureus*, total osteomyelitis incidence can be calculated from the data reported. This study found 4000 cases of osteomyelitis from a total of 1,351,362 nonobstetrical discharges; thus, osteomyelitis accounted for about 0.3% of the nonobstetrical discharges. Using the annual population estimates for the year and the counties studied (data.ny.gov), the overall incidence rate was 32.4 cases per 100,000 person-years.

Certain categories of osteomyelitis have received more investigative scrutiny. Incidence rates for acute hematogenous osteomyelitis in children have been published by multiple authors [9–15]. The time frames and populations studied varied among these studies, but there are common trends. In number of cases per 100,000 person-years, the incidence rates ranged from 1.31 in Norway (1990–1994) [11] to 82.5 for a Western Australian Aboriginal population (1971–1982) [9]. The incidence rate was found to be higher in males than females in all studies and was generally trending down over time. The incidence was lower for European populations (range 2–11.1 per 100,000 person-years) than for New Zealand Maori or Western Australian Aboriginal populations (range 29.1–82.5).

Incidence rates for prosthetic joint infections have also been reported by several groups, and, although covered in another chapter in this book, a few numbers are included here for reference. When calculated as rate of prosthetic joint infection (number of infections/total number of arthroplasty surgeries), the infection rate after primary knee arthroplasty ranges from 1% to 4%, and the rate after primary hip arthroplasty ranges from 1% to 2% [2, 16]. However, as these infection rates include all infections, not just cases of osteomyelitis, it is important to remember that the rate of osteomyelitis is likely much lower.

Open fractures represent a significant risk factor for osteomyelitis and infection rates after open tibia fractures have been reported from numerous studies. Incidence of infection can be estimated from the review of open fractures of the lower limb provided by Giannoudis and coauthors [17]. In this review, they report infection rate after open tibia fracture broken down by method of fracture fixation. The deep infection rates ranged from 35% for plate and screw fixation to 6.4% for reamed tibial nails. Not all studies reported cases of osteomyelitis, but from the ones that were reported, Giannoudis and coauthors determined that 4.2% of open tibia fractures treated with external fixation developed chronic osteomyelitis, whereas only 0.7% of the open tibia fractures treated with an undreamed tibial nail developed osteomyelitis. The annual incidence of open fractures has been estimated to be 30.7 per 100,000 person-years [18], with about 14% of open fractures occurring in the tibia. Thus, the incidence of open tibia fracture is about 4.3 cases per 100,000 person-years.

Finally, there are studies that report incidence rates for vertebral osteomyelitis. A study from Sweden [19] found an overall incidence of 2.2 per 100,000 person-years. *S. aureus* was found to be the causative agent in 34% of the cases, with *Mycobacterium tuberculosis* identified in 27% of the cases. A study from France [20] found an overall incidence of vertebral osteomyelitis

of 2.4 cases per 100,000 person-years. They found a strong correlation with age: there were 0.3 cases per 100,000 person-years in subjects under the age of 20 compared to 6.5 cases for subjects older than 70 years of age. They also found *S. aureus* (38%) and *M. tuberculosis* (31%) to be the most common causative bacteria.

7.3 General Complications

Complications can be divided into two groups: general or systemic and specific. General complications are associated with the systemic effects of any disease process, while specific complications are a result of the disease process itself. With osteomyelitis, the general complications that occur are usually related to the systemic effects of the inflammatory process caused by the infection – osteomyelitis. As osteomyelitis may either be an acute or chronic inflammatory process, these effects are manifested in different ways.

7.3.1 Systemic Sepsis

This condition usually occurs as a consequence of acute osteomyelitis which is untreated. Bacteremia occurs causing a septic state usually recognized by high temperature, increased pulse rate, and decreasing blood pressure. This is an emergency situation and demands rapid treatment for both the systemic condition and the local infective focus. Supportive treatment is the first response by providing intravenous fluids and pressors for hypotension as well as assessment for the need of respiratory support such as supplement oxygen or intubation depending on the severity of the pulmonary response to the ongoing inflammation. Antipyretic therapy may be needed depending on the severity of the hyperpyrexia. Once the resuscitation phase has commenced, empiric broadspectrum intravenous antibiotics based on the likely organism causing the osteomyelitis are the first line of treatment. If a prior bacterial diagnosis has been made, then specific antibiotics may be used as determined by the sensitivity results. Once the acute septic process has been identified and the antibiotic treatment commenced, it is imperative that the nidus of infection, usually a bone abscess, be addressed surgically with drainage. This will provide material for culture of the infecting organism to allow directed antibiotic therapy as well debridement of the infection removing as much as possible of the causative organisms and nonviable bone.

7.3.2 Chronic Disease Manifestations

Either untreated or under treated, acute osteomyelitis may progress to chronic osteomyelitis. As a result, the patient experiences a chronic inflammatory stimulus leading to a set of immune, physiological, metabolic, and behavioral responses for the patient. These responses usually lead to a state of fatigue or tiredness, malaise, occasionally nausea, and disinterest in life's activities. Chronic pain may develop at the site of the osteomyelitis leading to narcotic or analgesic abuse. The treatment of this situation rests with identification of the chronic nidus of infection and eradication of it. This may be only intravenous or oral antibiotics or surgical drainage and excision with reconstruction of the bone or amputation.

7.4 Specific Complications

7.4.1 Osteonecrosis

This is a rare complication usually seen in infants in the proximal femur. It is secondary to an infection that occurs in the metaphyseal region of the femoral neck prior to the appearance of the secondary ossification center of the femoral epiphysis. The infection will spread proximally across what would have been the epiphyseal plate into the cartilage anlage of the femoral head leading to destruction and potentially septic arthritis if it erupts into the hip joint. As the anlage for the femoral head is now deformed or destroyed, the child will have a deformity of the hip and sequent musculoskeletal disability as they grow. Treatment may be unpredictable due to the extent of damage to the epiphyseal analog.

7.4.2 Chronic Osteomyelitis

Chronic osteomyelitis is a situation that will develop with either untreated or under-treated acute osteomyelitis. The hallmark of this disease is dead bone with purulence surrounding it. The patient will usually present with a history of either acute osteomyelitis or some form of open bone injury or open fracture or prior operative manipulation of the bone with usually some form of implant placed. The patient will also complain of intermittent drainage from a sinus as well as fluctuating pain associated with swelling and erythema. While diagnosis is relatively straightforward, it is important to identify the necrotic dead bone or sequestrum that is perpetuating the osteomyelitis. Investigations include inflammatory markers such erythrocyte sedimentation rate (ESR), C reactive protein (CRP), white blood cell count (WBC), and blood cultures if the patient demonstrates evidence of febrile or systemic symptoms. Radiographic investigations include x-rays, CT scan, and/or

MRI to determine the extent of the intraosseous portion of the osteomyelitis and the nidus or sequestrum. The evaluation of the involucrum that is the new bone that has formed around the chronic infection is important as this will be part of the reconstructive process [6] (Fig. 7.1). Assessment of the patient's physiologic status is also important, as the ability to heal is extremely important in determining treatment and prognosis. Cierny and Mader [6] have described three host classes: normal, compromised, and prohibitive. A healthy host is not immunocompromised and has good vascularity around the site of infection. In a compromised host, there are local or systemic factors that compromise immunity and healing. Finally, a prohibitive host may have minimal disability but a poor prognosis for treatment and cure; in this case, the treatment is worse than the disease itself. The final aspect of the investigation and the first part of the treatment involves the harvesting of tissue for cultures from the involved area to identify the organism and its antibiotic sensitivities. Once the investigation is complete, surgical treatment is usually recommended. The surgical treatment involves eradication of the sequestrum by debridement, maintaining as much involucrum or living bone as possible to facilitate reconstruction and some method of skeletal support. Antibiotics based on the tissue culture sensitivities will be administered by the best route to achieve the maximum bone concentration [21]. Following eradication of the infection, bone reconstruction and deformity correction if needed can be undertaken. Amputation may also be recommended based on location, organisms, and chronicity [22].

Fig. 7.1 Anatomical classification of adult osteomyelitis. Type 1, intramedullary osteomyelitis; nidus is endosteal. Type II, superficial osteomyelitis; limited to bone surface. Type III, localized osteomyelitis; full thickness of cortex is involved. Type IV, diffuse osteomyelitis; entire circumference of the bone is involved [6]

Medullary

Superficial

Localized

Diffuse
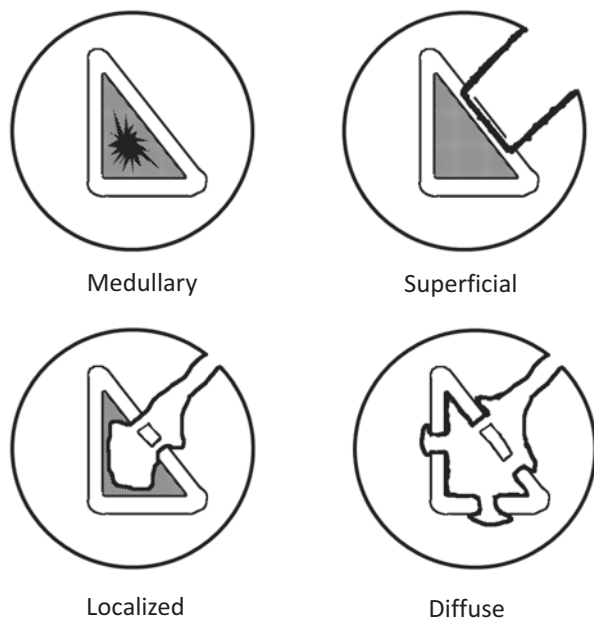
7.4.3 Subacute Osteomyelitis

This is an insidious condition with minimal symptoms of mild to moderate pain and a mildly elevated temperature. The etiology of this condition is poorly understood, but it may be related to increased host-pathogen relationship, administration of antibiotics before the onset of symptoms masking the full presentation, or decreased bacterial virulence. Delay in diagnosis is usually greater than 2 weeks. ESR is elevated in only 50% of patients and a pathogen only identified in 60% of cases. X-rays and bone scanning techniques are usually positive. It normally occurs in the metaphyseal region of the bone and is recognized through a radiolucent area surrounded by sclerotic borders which may cross into the epiphysis or be metaphyseal or cortical in location. It can have multiple cavities, but there will be no true destruction of cortical bone. The classic example of subacute osteomyelitis was described by Brodie in 1836 [23] as a localized abscess in a patient's tibial metaphyseal region that had no prior history of any infection. Treatment usually involves surgical biopsy for culture and curettage followed by antibiotics.

7.4.4 Chronic Recurrent Multifocal Osteomyelitis

This is an unusual autoinflammatory condition in children and adolescence with an insidious onset of pain and signs of inflammation occurring at multiple bony sites usually localized to the metaphysis or epiphysis. The following have been proposed as criteria for the diagnosis of CRMO: two or more bone lesions mimicking osteomyelitis, radiographic and bone scan findings consistent with osteomyelitis, 6 months or more of chronic and relapsing symptoms, failure of response to at least 1 month of appropriate therapy, and a lack of other identifiable cause. There is no effective treatment, but bisphosphonates have been shown to be helpful [24].

7.4.5 Sclerosing Osteomyelitis of Garré

This chronic condition described by Garré in 1893 [25] in young children and adolescences is noted to have thickened and distended cortical bone with no abscesses or sequestra. Its cause is unknown but could be a low-grade infection with an anaerobic bacterium such as *Propionibacterium acnes* or an infection such as actinomycosis. The patient will have intermittent pain, swelling, and tenderness over the involved bone, and the bone itself will be expanded in its cortical regions with sclerosis. The ESR and CRP levels are mildly elevated, and a biopsy will demonstrate chronic low-grade nonspecific infection. No treatment has been successful, but fenestration of the involved bone and broad-spectrum antibiotics have been helpful in some cases [26].

7.4.6   Malignant Transformation in Chronic Osteomyelitis (Marjolin's Ulcer)

Malignancy can arise from chronic osteomyelitis at a rate of 1.6–23% most commonly seen in areas with limited access to medical care [27–29]. This tumor is proposed to result from chronic inflammation stimulating stem cells resulting in cancer [30, 31]. It is seen most frequently in males 18–40 years with the malignant transformation occurring over a latent period of 18–72 years. The tibia is the most frequently affected bone [32]. Most of the tumors are aggressive squamous cell carcinomas arising from a sinus tract, but basal cell carcinomas, reticulum cell carcinoma, fibrosarcoma, and others have been reported. Clinical presentation is increased pain and foul-smelling drainage associated with erythema, bleeding, and an enlarging mass. Radiographs will demonstrate periosteal changes and progressive bone destruction, while a CT scan will show bone extent and the MRI is used to assess soft tissue involvement. A high degree of clinical suspicion associated with biopsy of a chronic draining sinus or poorly healing ulcers associated with chronic osteomyelitis generally will be needed to make the diagnosis. These are aggressive malignancies with a tendency for local recurrence and lymph node metastases. Amputation is the most reliable means of treating osteomyelitis associated with malignant change although wide local resection (Mohs procedure) in patient with localized non-metastatic disease is possible [33].

7.4.7   Amputation

Amputation may be considered a major complication of osteomyelitis. In the acute stage associated with overwhelming sepsis, it may be a lifesaving procedure. In the chronic osteomyelitis, amputation may be a life-changing procedure ridding the patient of a site of chronic inflammation. This is indicated in situations in which there has been recalcitrant osteomyelitis with multiple different organisms, multiple surgeries, deformity, nonunion, or malignant transformation.

7.4.8   Deformity

Deformity can occur either through acute osteomyelitis in the growing child, secondary to chronic osteomyelitis, or osteomyelitis secondary to internal fixation or an operative bone procedure. The deformity may need to be corrected and maybe complicated by the fact that there is a chronic ongoing infection that requires eradication before corrective surgery.

7.4.9 Fracture

Fracture is not an uncommon complication during the process of treating osteomyelitis. Bone strength and integrity maybe compromised as a result of the debridement necessary to rid the patient of the infected nidus, leading to the fracture. Avoidance of this complication requires some form of protective treatment such as the use of casts, external fixation, or internal fixation depending on the circumstance and severity of the infection. If a fracture occurs, it becomes more difficult to treat because it coincides with the site of the infection. If the infection is still active, then usually some form of temporary fracture fixation (e.g., external fixation) will be required until the infection is eradicated, at which point internal fixation and bone grafting if necessary can be carried out. Should the fracture occur after the infection has been eradicated, standard operative or nonoperative fracture fixation can be carried out as indicated [22].

7.5   Innovation in Osteomyelitis Treatments: Pediatric

Pediatric osteomyelitis develops in the metaphysis of long bones and is thought to be due to tortuous blood flow. Infection may involve intraosseous, subperiosteal, extraperiosteal abscesses or extend to a joint, particularly in joints where the capsule is intra-articular. The three most common sites for osteomyelitis in children are femur, tibia, and humerus [34].

Early diagnosis of pediatric osteomyelitis is key to urgent initiation of appropriate treatment. Conventional radiographs only show evidence of bone destruction 1–2 weeks after infection begins in children with osteomyelitis. Therefore, utilization of advanced imaging must be employed to achieve early identification of osteomyelitis. Treating physicians must consider cost, accuracy, and potential delay in treatment or diagnosis when selecting advanced imaging. Ultrasound is a low-cost method to evaluate for joint effusion, abscesses, and DVT but allows limited visualization of osteomyelitis bone involvement. Though CT provides better imaging of the bone, particularly sequestration, it has limited use in pediatrics due to radiation exposure [34]. Bone scintigraphy, though highly sensitive for early diagnosis of osteomyelitis, has low specificity and cannot distinguish between soft tissue, joint, and bone infection. For these reasons, MRI has increasingly come into favor for evaluation of osteomyelitis. MRI can be utilized to identify soft issue abscess, myositis, joint effusion, and osteomyelitis including intraosseous and subperiosteal abscesses. The major limitations to MRI have been timely access to prevent delay in treatment and the possible need for general anesthesia. The creation of MRI protocols at institutions greatly decreases the length of time needed for the MRI and therefore may negate the need for anesthesia [34, 35]. Protocols use limited series (coronal STIR, coronal T1, axial T2 fat-suppressed, post-contrast coronal, and axial T1 fat-suppressed) to allow for quicker identification of location and extent of

involvement of infection (Fig. 7.2). Studies have also suggested adding MRI to the workup of septic arthritis in children given the high incidence of concomitant osteomyelitis. In evaluation for septic hip, the utilization of MRI identified osteomyelitis in 47.9% of patients with three or four positive Kocher criteria [36]. Institutions have also created clinical practice guidelines to expedite MRIs and create pathways to have daily availability of MRI for add-on patients under general anesthesia and facilitate the ability to go directly to the operating room from the MRI [35].

Antibiotic therapy is the gold standard with initial empiric therapy based on the patient's age. Traditionally, intravenous antibiotic therapy for acute osteomyelitis was of 6 weeks' duration. This was associated with prolonged hospital stays, high costs, and need for central venous access. These long courses of intravenous antibiotic therapy had reported complication rates of 25–38% and 19–27% rehospitalization rate [34]. The current trend has shifted to a short course of intravenous antibiotics with early conversion to oral antibiotics. Several studies have shown efficacy of treating acute osteomyelitis with only a few days of intravenous antibiotics followed by oral antibiotics for 3 to 4 weeks. Transition to oral antibiotics is guided by apyrexia, an improvement in the patient's condition and reduction in CRP. Complicated cases of acute osteomyelitis involving neonates, immunocompromised patients, or bacteria such as methicillin-resistant *S. aureus* (MRSA) or *Salmonella* still require longer course of intravenous antibiotics [37]. In addition to shortened courses of intravenous antibiotics, measures including development of evidence-based clinical practice guidelines and a classification system for the severity of illness in acute hematogenous osteomyelitis have been associated with shorter hospital stay and decreased readmissions [35, 38].

Surgical management may be necessary as an adjunct to antibiotic therapy in children with acute hematogenous osteomyelitis. Specific techniques or extent of surgery for pediatric osteomyelitis have not been clearly defined. Surgical treatment

Fig. 7.2 Coronal STIR image of a 12-month-old female presenting with left arm pain and swelling. MRI shows osteomyelitis of humeral shaft with lateral subperiosteal abscess

can vary from minimally invasive needle bone biopsy or aspiration of abscess to extensive cortical bone window with debridement of cancellous bone [34] (Figs. 7.3a and 7.3b). Children with joint effusion and/or large abscess should be considered for immediate surgical intervention. Children with moderate subperiosteal or intraosseous abscesses that fail to respond to antibiotics after 48–72 hours are candidates for surgery. Any surgery for osteomyelitis should involve a bone biopsy to rule out malignant process and cultures. Decisions for repeat surgical intervention are based on child's fever curve and CRP trend [34].

In children, it is recognized that there is a significant rate of osteomyelitis with concurrent adjacent septic arthritis of 17–33% [34, 39]. The cause of these concurrent joint infections is the spread of osteomyelitis into the joint, particularly in joints were the metaphysis is intracapsular. Risk factors for concurrent infections include age (newborns and adolescents), shoulder infections, increased duration of symptoms before presentation, and *Staphylococcus aureus*, both methicillin-susceptible *S. aureus* (MSSA) and MRSA, infection. Recognizing concurrent infection is important as these patients have more severe illness associated with increased hospital stay, more surgeries, and more intensive care unit (ICU) admissions [39]. Complications of missed osteomyelitis include untreated infection, avascular necrosis of the bone, and pathologic fracture. Given the significant rate of concurrent osteomyelitis with hip septic arthritis, studies have purposed performing femoral neck aspiration during the incision and drainage (I&D) of the septic hip, which was noted to have increased sensitivity and specificity for identifying osteomyelitis, compared to MRI (Figs. 7.4a and 7.4b). Seeding of the bone and fracture at aspiration site were not noted [40]. Humeral osteomyelitis has also been associated with both shoulder and elbow septic arthritis [41]. A high rate of osteomyelitis associated with shoulder septic arthritis in children has been noted with 75% of children obtaining an MRI for septic arthritis found to have osteomyelitis with 26% of those having a subperiosteal abscess. The MRI evaluation guided surgical treatment to include subperiosteal abscess drainage or corticotomy at the time of I&D of the shoulder [42]. As osteomyelitis typically requires a longer duration of antibiotics than septic arthritis alone and may require surgical intervention beyond I&D of the involved joint, it is key for the physician to evaluate for and identify possible bone involvement with septic joint.

Fig. 7.3a Sagittal STIR MRI of a 15-month-old male that presented with refusal to bear weight on the left foot and elevated ESR and CRP. MRI shows a Brodie's abscess of the talar head
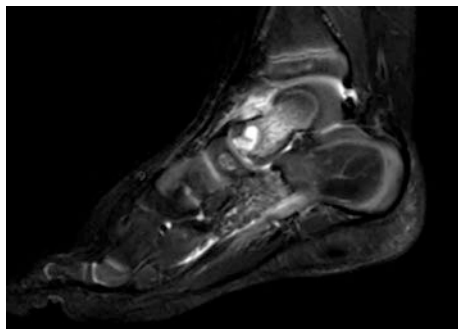
Fig. 7.3b Intraoperative image of percutaneous aspiration of Brodie's abscess and bone biopsy

Fig. 7.4a Axial fat suppressed T2 image of a 12-year-old with history of sickle cell anemia that presented with right hip pain and elevated WBC, ESR, and CRP. MRI showed large hip effusion and femoral head osteomyelitis
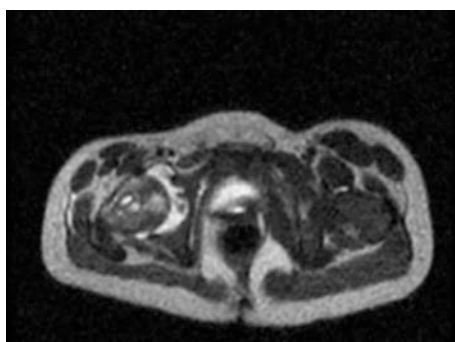
The overall rate of complications from acute osteomyelitis in children is approximately 6%. Complications include chronic osteomyelitis rate of 1.7%, recurrent infection rate of 6.8%, deep vein thrombosis (DVT) rate of 0.4–6%, and pathologic fracture rate of 1.7% [34]. Early identification of osteomyelitis and appropriate, directed treatment can decrease these risks in the pediatric population.

7.6   Innovations in Osteomyelitis Treatments: Adults

The management of adult osteomyelitis remains based on the principle developed by Hiram Winnett Orr when he stated that all necrotic bone must be removed with saucerization of the resulting cavity followed by immobilization of the bone [43]. Recently this principle has included innovative advances in the diagnosis of necrotic bone, management of the dead space, and judicious appropriate use of antibiotics.

Fig. 7.4b Intraoperative image of femoral neck needle aspiration performed during the right hip I&D
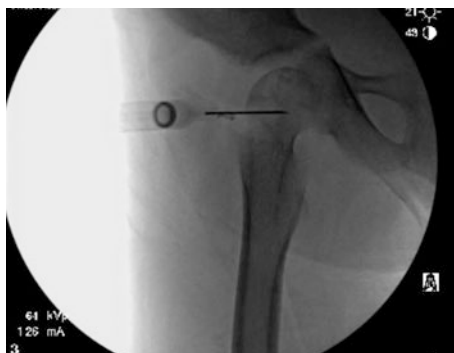
Osteomyelitis may occur either as acute or chronic. Acute osteomyelitis in the adult is uncommon and is usually related to hematogenous spread from a remote infective nidus. It is associated with the classic signs and symptoms of an acute inflammatory process: rubor (redness), calor (heat), tumor (swelling), dolor (pain), and functio laesa (loss of function). Acute osteomyelitis is usually monomicrobial and most commonly caused by a coagulase-positive organism, specifically *Staphylococcus aureus*. The management consists of rapid identification and diagnosis, prompt surgical debridement, and antibiotics based upon cultures.

Chronic osteomyelitis, however, is more common in the adult and is usually non-hematogenous in spread occurring from some form of continuous contiguous focus either from surgical inoculation from the use of implants or secondary to vascular insufficiency or neuropathies which allows for skin breakdown and colonization. Chronic osteomyelitis is usually polymicrobial, again with *Staphylococcus aureus* being the most commonly identified organism, but strains of *Streptococcus*, *Bacteroides*, *Klebsiella*, *Enterococcus*, and *Pseudomonas* have also been identified.

As the most common etiology of adult osteomyelitis is secondary to some type of inoculation of bacteria through trauma, disease, or surgery, prevention becomes the most critical component of the management. Recognition of this fact has led to the better patient assessment prior to elective surgery particularly in arthroplasty. Recognition of the importance of rapid delivery of antibiotics in open fractures and appropriate debridement techniques as well as systems to improve the management of diseases such as diabetes and arteriosclerosis has decreased the incidence of osteomyelitis. Finally, the standardization and mandatory use of prophylactic pre-operative antibiotics has also helped to decrease the incidence of infection in implant-related surgery, and hence the potential for osteomyelitis has decreased.

The recognition of the ability of an infecting bacterium to create its own protective environment – the biofilm – during the chronic process has been a stimulus to management innovations [44]. The body's natural response to the implant is the formation of a film of adhesion factors such as fibronectin collagen binding proteins around the implant. The infecting bacteria modify this membrane and become encased in it, protecting the bacteria from phagocytosis by the white blood cells as well as secreting factors that decrease bone formation by increasing osteoblast

apoptosis and the expression of receptor activator of nuclear kappa-B ligand (RANKL), leading to decreased bone formation. The biofilm is also hydrophobic which inhibits the penetration of antibiotics, and biofilm bacteria may become sessile and dormant thus not susceptible to many antibiotics. Finally, due to the breakdown of cells and bacteria, biofilm bacteria are able change their genetic makeup and potentially become antibiotic resistant. This understanding of the presence of the biofilm has led to the understanding of why it is so difficult to clear the infection and the understanding in certain circumstances that a symbiotic relationship between the sessile bacteria, the biofilm, and the host may be acceptable.

7.6.1 Innovations in Diagnosis

Chronic osteomyelitis presents clinically as a chronic vague illness with a low-grade temperature, recurrent sinus tract and drainage, and some past history of either trauma, surgery, or an implant.

Laboratory investigation usually involves an assessment of the inflammatory process through the use of ESR and CRP levels in addition to white blood cell counts. These are not extremely helpful for a specific diagnosis as they are nonspecific tests for inflammation but are needed for following the effect of therapy. As the CRP measures the digestion products of substances foreign to the host and hence will be elevated when bacteria are in the systematic circulation, the ESR only measures elevation of serum protein from any cause [45]. Newer blood tests involving interleukin 6 and procalcitonin have not been shown to any better diagnostically than the use of CRP [6].

7.6.2 Cultures and Culture-Independent Methods

It is now recognized that cultures should be tissue and obtained from the site of the inflammatory process. Swabs from a draining sinus or local superficial tissue are not predictive of the infecting organism(s) [46]. Recently, newer methods to better identify the presence of infection and organisms have been introduced. The polymerase chain reaction (PCR) test has improved the ability to identify bacteria that are difficult to isolate by traditional methods, particularly slow-growing bacteria, which may improve identification of causative organisms in culture-negative cases of osteomyelitis [47]. While there is concern that with breakdown of bacteria, bacterial DNA contamination can be taken as a false-positive test, it is not clear that DNA from dead bacteria remain detectable for longer than 24–48 hours [48]. The introduction of pathogen-specific PCR is now faster and more specific allowing improved identification of an infection and the organism(s) causing it [49]. Fluorescence in situ hybridization (FISH) is another molecular biologic nucleic acid-based technique to assess DNA and RNA that has been recently applied to osteomyelitis in the

rabbit [50]. Matrix-assisted laser desorption-ionization coupled with time of flight analysis mass spectroscopy (MALDI-TOF/MS) uses a soft laser ionization of the intact bacteria or its extract to identify specific bacteria's unique surface proteins and peptides. This test has the potential to provide a cheap reliable and efficient method for diagnosis [51, 52]. Finally, PCR electron spray ionization with mass spectrometry (PCR-ESI/MS) has been used to identify infection, the pathogen, and resistance markers in prosthetic joint infection [53].

7.6.3   Imaging

The simplest and cheapest way to diagnose osteomyelitis remains plain radiographs [45]. These show periosteal reaction, cortical erosions, focal osteopenia, osteolysis, and endosteal scalloping, as well as the involucrum which is new bone formation around the nidus of infection (the sequestrum) and the cloaca that is the opening through the involucrum to allow drainage of purulent material.

CT scan with coronal and sagittal reconstructions provides the most detailed knowledge of bony involvement best used for staging osteomyelitis. Implants will tend to distort the image to some degree, but recent improvements in the technique have minimized this problem. MRI will differentiate between soft tissue and bone marrow involvement as well as early detection of acute osteomyelitis. Intravenous gadolinium contrast enhances the differentiation between vascularized infected areas and nonvascular areas such as the sequestrum. Implants, recent surgery, and scar tissue can limit the ability of the MRI to detect infection.

The three-phase bone scan using ^{99m} technetium methylene diphosphonate is used to determine bone perfusion and bone turnover; unfortunately tumors, fracture healing, as well as infection will give positive scans. The first phase between 0 and 60 seconds assesses blood flow, the second phase from 2 to 5 minutes shows the blood pool, and the third phase from 2 to 4 hours demonstrates static bone metabolism. If phase one and two indicate increased uptake of isotope with phase 3 showing no uptake, soft tissue infection is likely while increased uptake in all three phases may be a nonspecific indicator of osteomyelitis. Indium-111-labeled white blood cells will infiltrate into an inflamed area such as osteomyelitis. In order to determine a positive test, it is necessary to perform two sets of images at 3 to 4 hours and 20 to 24 hours. If the uptake is increasing in both sets of images, then an inflammatory process such as osteomyelitis is likely, while a decrease or steady uptake at 20 to 24 hours implies no infection. In chronic osteomyelitis, the sensitivity and specificity are low and hence are not indicated to use [54, 55].

More recent innovations have led to the combination of radiologic tests with nuclear medicine procedures to increase the diagnostic value. Single-photon emission computed tomography has been combined with the triphasic and WBC scan (SPECT-CT), while another more common combination is ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET/CT). Although FDG-PET/CT is not routine at the present time, this technique has shown a sensitivity of 86–94% and specificity of 76–100% [54].

7.6.4 Treatment

As mentioned, the basis for treatment rests on eradication of all dead and infected bone based on the clinical situation. To determine if possible and how best to accomplish this, a plan must be developed. This is accomplished by assessment of the host patient and the location and extent of the infective process. A significant advance in the planning of treatment is a result of the classification of the host and boney extent provided by Mader and Cierny [6]. The host has three basic levels. A type A host is a healthy individual who is immunocompetent and has excellent local vascular and viable tissue. The type B host has conditions that will compromise their immune response and healing potential. These conditions may be local (type B-local) such as prior trauma or surgery, chronic sinus, or poor soft tissue coverage, all of which affect the local vascular and vitality of the infected site. The other type B host has systemic conditions (type B-systemic) that will make healing difficult such as immune compromise, malnutrition, or diabetes. It is evident that both the local and systemic condition will need to be corrected in order to maximize the treatment result. The type C host are those who are too compromised to basically undertake and consider surgical management for the treatment as it may be worse than the living with the disease itself.

To improve the type B-local patient's response to treatment, a thorough evaluation of the local soft tissues is required followed by a plan to rectify these problems. The chronic draining sinus is usually associated with scarred skin and subcutaneous tissue which must be dealt with by excision. A sinus may directly communicate with the sequestrum or may have a circuitous route through fascial planes to the exterior. By excising the sequestrum the sinus will usually disappear. Scarred skin, subcutaneous tissue, and muscle that has been debrided must be replaced with viable tissue. The aim is to achieve a clean, infection-free, and viable bed to allow regeneration of the bone and functional soft tissues if possible. Consultation with a plastic or microvascular surgeon in order to provide some form of viable soft coverage is usually required. Free vascularized musculocutaneous flaps are better than fasciocutaneous flaps as they provide a better blood flow to the area and better phagocytosis if any bacteria may be under the flap [56–59].

The management of the boney lesion begins with assessment of the radiographic investigations to determine the extent of the osteomyelitis. Usually the plain radiographs and CT scan are all that are needed to determine the location of sequestrum and the extent of bone involvement particularly the involvement of the metaphysis and articular surfaces. The MRI tends to overestimate the bone involvement but is very helpful in assessing the presence of noncontiguous lesions known as "jump lesions" and intramedullary osteomyelitis [45]. The location and extent of bone involvement as defined by Cierny will guide the surgical planning [6]. A stage 1 lesion involves only the medullary canal and will be adequately treated by intramedullary debridement and removal of any implant if its function is impaired. The recent introduction of the reamer-irrigator-aspirator reaming system has greatly facilitated the ability to debride the intramedullary canal as the purulent material

can be removed by suction and not pushed forward by a reamer to be deposited in another location in the medullary canal [60, 61]. A stage 2 lesion is uncommon and involves only the superficial periosteal region of the cortex with no medullary spread. This is caused by local colonization secondary of a chronic wound. Management consists of removal of the infected bone until viable bleeding bone is seen, and then the chronic wound is managed by an appropriate soft tissue procedure to enhance the vascularity. A stage 3 lesion involved the full thickness of the cortex and endosteum and implies intramedullary spread, but there is non-infected bone in the same region. This is a result of direct bone trauma causing devascularization of the bone and invasion of the bone with bacteria. The management requires removal of the infected bone, but as there is healthy non-infected bone in the same area, skeletal stability is usually not affected. The final lesion is stage 4, which is a permeative lesion involving a segment of the bone. As this segment is completely devascularized and infected, a complete excision of the infected segmental region is necessary. This leads to axial skeletal instability which will need to be addressed as well as the created dead space.

As with understanding for the need for a viable soft tissue envelope, the management of the infected bone follows a similar plan. All dead and nonviable bone must be removed. This will leave the surgeon with a bone defect that must be managed. Following excision of the infected region, the involved bone will either maintain its axial stability or there is a segmental defect leading to axial instability. The major innovations in the treatment of osteomyelitis are seen in the area of management – eradication of infection, dead space management, and bone reconstruction.

The initial surgical treatment involves the removal of all dead bone and obtaining a biopsy for pathology and culture. Many attempts to define viable bone have been tried, but to date the best is the surgeon's estimate of the bone viability by observation of a punctate cortical bleeding site known as the paprika sign. After removal of the dead bone, the dead space remaining is managed so as to allow for the development of a viable vascular bed and at the same time locally eradicate any remaining infective organisms. In stage 1, intramedullary osteomyelitis, the debrided medullary canal may be sterilized with insertion of polymethylmethacrylate (PMMA) antibiotic-coated nail for 6 to 8 weeks [61] or the implantation of antibiotic impregnated biodegradable substances such as calcium sulphate or calcium phosphates (Fig. 7.5). These compounds will deliver the antibiotics and at the same time be resorbed or incorporated into the bone avoiding a second operation for removal. There are no commercially available biodegradable antibiotic delivery systems approved in the USA, but the surgeon may mix the antibiotic with the biodegradable substances. Reported results show up to 86% healing with these compounds [62, 63]. There is a problem of operative site drainage particularly with some preparation of calcium sulphate. Bioactive glass (BAG) S53P4 has been shown both in vitro and in vivo to be as effective as calcium-based substitutes with less drainage. A similar management plan for stage 2 lesion can be done. Following debridement and saucerization of the bone, an antibiotic impregnated resorbable delivery device such as calcium sulfate can be applied and covered with viable soft tissue [64, 65].

Fig. 7.5 Radiographic appearance of antibiotic-eluting resorbable calcium sulfate beads

Stage 3 and 4 lesions will have local antibiotics placed in the created defect or dead space. As these stages will usually require several surgeries for bone reconstruction, the antibiotic delivery agent has been PMMA impregnated with antibiotics such as vancomycin or tobramycin in the form of beads or a block [61, 66]. As PMMA forms with an exothermic reaction, only heat-stable antibiotics may be mixed with it. These antibiotics are generally empirically chosen until the cultures reveal the specific bacteria. The PMMA has been found to stimulate a foreign body reaction in the dead space. After 6 weeks a membrane has formed that is highly vascularized and rich in bone-forming genes and cytokines. This is ideal for the placement of a bone graft to assist in the bone reconstruction [67] (Fig. 7.6).

In cases where there is no axial stability or that after debridement the surgeon is concerned that the remaining viable bone is not strong enough to withstand physiological loads, some form of stability will be needed. This is dependent on the stage of treatment. If there is ongoing infection or during the initial phase of infection eradication, the usual device for obtaining stability is an external fixator as it assures no foreign material in the infected region. Once the infection is eradicated, then the

Fig. 7.6 (**a**) A 44-year-old male sustained this open tibial shaft fracture with significant soft tissue compromise. He underwent urgent irrigation and debridement, (**b**) intramedullary nailing, and free flap soft tissue coverage. (**c**) He developed a wound infection 1 month later, with cultures that grew MRSA. He was treated with irrigation and debridement and IV antibiotics. (**d**) Due to inability to clear the infection, he underwent hardware removal with placement of an antibiotic cement rod. (**e**) Eight weeks later, inflammatory markers had returned to normal, and he was treated with removal of the antibiotic rod and exchange

(continued)
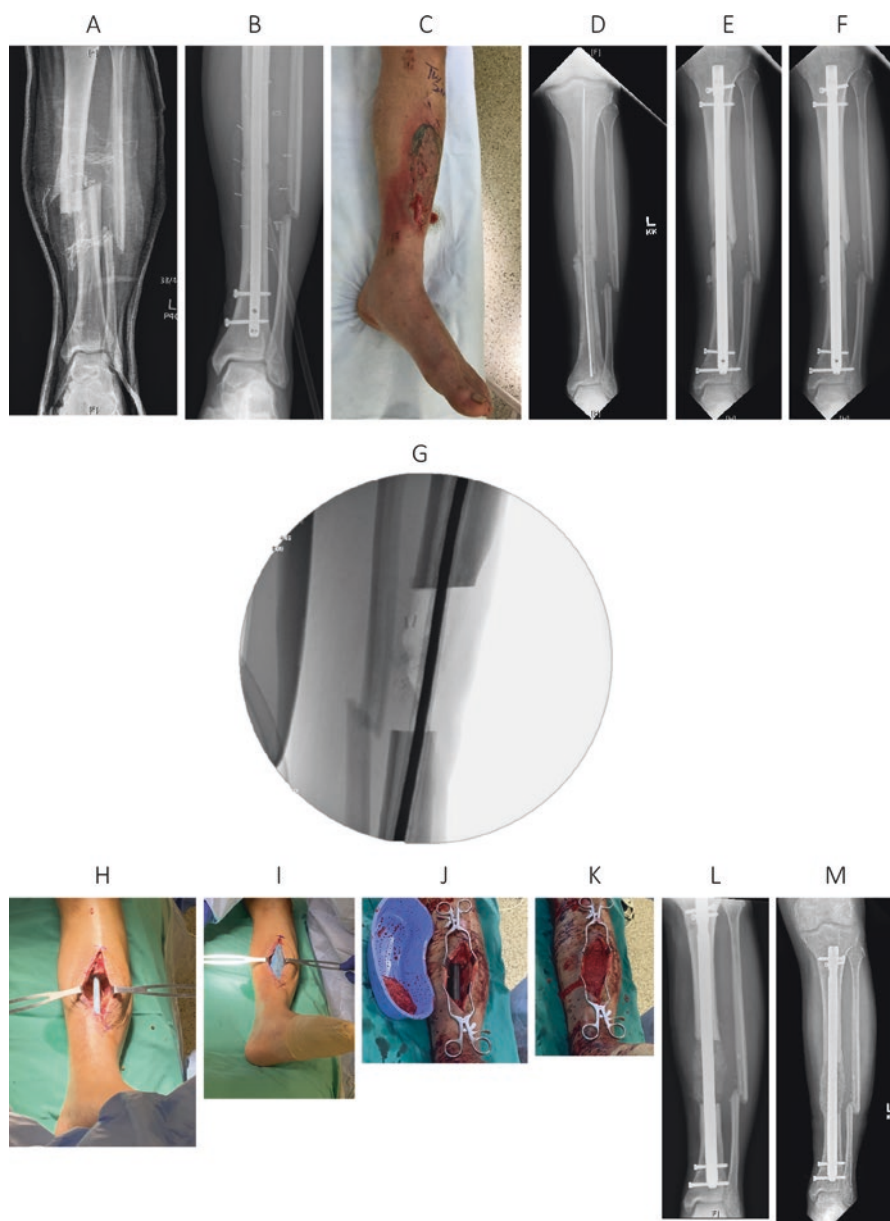
nailing of the tibia. (**f**) Five months later, the patient returned with chronic drainage and radiographic signs of osteomyelitis. (**g**) A wide resection of involved tibial bone was performed, (**h**) with placement of an antibiotic rod and a (**i**) antibiotic cement spacer. He was treated with IV antibiotics, and 8 weeks later, he returned to the operating room for removal of the cement spacer and rod and (**j**) repeat intramedullary nailing and (**k**) iliac crest bone grafting to the defect. (**l**) Immediate postoperative x-rays and (**m**) 6-month follow-up. The patient was healed with no pain and no issues referable to his leg

use of internal fixation is appropriate. This is usually in the form of an intramedullary nail for diaphyseal lesion and plate fixation metaphyseal or articular involvement. The use of distraction osteogenesis is another technique that can be used to provide both stability and regeneration of the bone. This was original described by Ilizarov, but recent advances in computerized direct transport and deformity correction are available. Recently the introduction of a motorized intramedullary nail has offered a more patient acceptable device to achieve a similar result.

7.6.5   Antibiotics

Antibiotics should be used in an appropriate well-defined protocol agreed upon by both the treating surgeon and infectious disease specialist. Their long-term use is probably no longer expected in healthy hosts (type A and type B-local) [68, 69]. It is usually possible to give these patients a 3- to 5-day parenteral course of antibiotics followed by oral antibiotics for 7 to 10 days [45].

Antibiotics have also been used long term to suppress infection long enough to allow for bony union in the setting of fractures, for example, in cases where you may need to leave the metal implant in place. Once the fracture is healed, the antibiotic therapy can be stopped and the implant can be removed. It should be realized that using antibiotic suppressant therapy is not a cure. It should be used on an interim basis to deal only with those situations where the infection flares up. It should be a non-broad spectrum and as specific as possible to treat the specific bacteria for 7 to 10 days. The idea is to be able to eliminate the planktonic systemic bacteria to allow the biofilm to stop releasing bacteria. Essentially the patient and the biofilm live in symbiosis and only when this relationship is disrupted are antibiotics needed. This avoids the complications of long-term antibiotic and the increasing development of resistant organisms [45].

References

1. Floyed RL, Steele RW. 2003. Culture-negative osteomyelitis. Pediatr Infect Dis J. 22(8):731–736.
2. Kalbian I, Park JW, Goswami K, et al. 2020. Culture-negative periprosthetic joint infection: prevalence, aetiology, evaluation, recommendations, and treatment. Int Orthop. 44(7):1255–1261.
3. Waldvogel FA, Medoff G, Swartz MN. 1970. Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects. N Engl J Med. 282(4):198–206.
4. Waldvogel FA, Medoff G, Swartz MN. 1970. Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects (second of three parts). N Engl J Med. 282(5):260–266.
5. Waldvogel FA, Medoff G, Swartz MN. 1970. Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects. 3. Osteomyelitis associated with vascular insufficiency. N Engl J Med. 282(6):316–322.

6. Cierny G, 3rd, Mader JT, Penninck JJ. 2003. A clinical staging system for adult osteomyelitis. Clin Orthop Relat Res 414:7–24.

7. Kremers HM, Nwojo ME, Ransom JE, et al. 2015. Trends in the epidemiology of osteomyelitis: a population-based study, 1969 to 2009. J Bone Joint Surg Am. 97(10):837–845.

8. Rubin RJ, Harrington CA, Poon A, et al. 1999. The economic impact of Staphylococcus aureus infection in New York City hospitals. Emerg Infect Dis 5(1):9–17.

9. Gillespie WJ. 1985. The epidemiology of acute haematogenous osteomyelitis of childhood. Int J Epidemiol 14(4):600–606.

10. Craigen MA, Watters J, Hackett JS. 1992. The changing epidemiology of osteomyelitis in children. J Bone Joint Surg Br 74(4):541–545.

11. Dahl LB, Høyland AL, Dramsdahl H, Kaaresen PI. 1998. Acute osteomyelitis in children: a population-based retrospective study 1965 to 1994. Scand J Infect Dis 30(6):573–577.

12. Blyth MJ, Kincaid R, Craigen MA, Bennet GC. 2001. The changing epidemiology of acute and subacute haematogenous osteomyelitis in children. J Bone Joint Surg Br. 83(1):99–102.

13. Rossaak M, Pitto RP. 2005. Osteomyelitis in Polynesian children. Int Orthop. 29(1):55–58.

14. Malcius D, Trumpulyte G, Barauskas V, Kilda A. 2005. Two decades of acute hematogenous osteomyelitis in children: are there any changes? Pediatr Surg Int. 21(5):356–359.

15. Riise Ø R, Kirkhus E, Handeland KS, et al. 2008. Childhood osteomyelitis-incidence and differentiation from other acute onset musculoskeletal features in a population-based study. BMC Pediatr 8:45.

16. Koh CK, Zeng I, Ravi S, et al. 2017. Periprosthetic Joint Infection Is the Main Cause of Failure for Modern Knee Arthroplasty: An Analysis of 11,134 Knees. Clin Orthop Relat Res 475(9):2194–2201.

17. Giannoudis PV, Papakostidis C, Roberts C. 2006. A review of the management of open fractures of the tibia and femur. J Bone Joint Surg Br. 88(3):281–289.

18. Court-Brown CM, Bugler KE, Clement ND, et al. 2012. The epidemiology of open fractures in adults. A 15-year review. Injury. 43(6):891–897.

19. Beronius M, Bergman B, Andersson R. 2001. Vertebral osteomyelitis in Göteborg, Sweden: a retrospective study of patients during 1990–95. Scand J Infect Dis. 33(7):527–532.

20. Grammatico L, Baron S, Rusch E, et al. 2008. Epidemiology of vertebral osteomyelitis (VO) in France: analysis of hospital-discharge data 2002–2003. Epidemiol Infect. 136(5):653–660.

21. Rao N, Ziran BH, Lipsky BA. 2011. Treating osteomyelitis: antibiotics and surgery. Plast Reconstr Surg. 127 Suppl 1:177s–187s.

22. Roesgen M, Hierholzer G, Hax PM. 1989. Post-traumatic osteomyelitis. Pathophysiology and management. Arch Orthop Trauma Surg. 108(1):1–9.

23. Brodie BC. 1836. Pathological and surgical observations on disease of joints.

24. Roderick MR, Shah R, Rogers V, et al. 2016. Chronic recurrent multifocal osteomyelitis (CRMO) – advancing the diagnosis. Pediatr Rheumatol Online J. 14(1):47.

25. Garre C. 1893. Uber besondere Formen und Folgezustande der akuten infektiosen Osteomyelitis. Beitr z klin Chir. 10:241–298.

26. Song S, Jeong HJ, Shin HK, et al. 2019. Sclerosing osteomyelitis of Garré: A confusing clinical diagnosis. J Orthop Surg (Hong Kong). 27(3):2309499019874704.

27. Altay M, Arikan M, Yildiz Y, Saglik Y. 2004. Squamous cell carcinoma arising in chronic osteomyelitis in foot and ankle. Foot Ankle Int. 25(11):805–809.

28. Kerr-Valentic MA, Samimi K, Rohlen BH, et al. 2009. Marjolin's ulcer: modern analysis of an ancient problem. Plast Reconstr Surg. 123(1):184–191.

29. Onah, II, Olaitan PB, Ogbonnaya IS, Onuigbo WI. 2006. Marjolin's ulcer (correction of ulcer) at a Nigerian hospital (1993–2003). J Plast Reconstr Aesthet Surg. 59(5):565–566.

30. Sell S. 2011. Infection, stem cells and cancer signals. Curr Pharm Biotechnol. 12(2):182–188.

31. Samaras V, Rafailidis PI, Mourtzoukou EG, et al. 2010. Chronic bacterial and parasitic infections and cancer: a review. J Infect Dev Ctries. 4(5):267–281.

32. Alami M, Mahfoud M, El Bardouni A, et al. 2011. Squamous cell carcinoma arising from chronic osteomyelitis. Acta Orthop Traumatol Turc. 45(3):144–148.

33. Panteli M, Puttaswamaiah R, Lowenberg DW, Giannoudis PV. 2014. Malignant transformation in chronic osteomyelitis: recognition and principles of management. J Am Acad Orthop Surg. 22(9):586–594.

34. Funk SS, Copley LA. 2017. Acute Hematogenous Osteomyelitis in Children: Pathogenesis, Diagnosis, and Treatment. Orthop Clin North Am. 48(2):199–208.

35. Copley LA, Kinsler MA, Gheen T, et al. 2013. The impact of evidence-based clinical practice guidelines applied by a multidisciplinary team for the care of children with osteomyelitis. J Bone Joint Surg Am. 95(8):686–693.

36. Nguyen A, Kan JH, Bisset G, Rosenfeld S. 2017. Kocher criteria revisited in the era of MRI: How often does the Kocher Criteria identify underlying osteomyelitis? J Pediatr Orthop. 37(2):e114-e119.

37. Castellazzi L, Mantero M, Esposito S. 2016. Update on the management of pediatric acute osteomyelitis and septic arthritis. Int J Mol Sci. 17(6).

38. Athey AG, Mignemi ME, Gheen WT, et al. 2019. Validation and modification of a severity of illness score for children with acute hematogenous osteomyelitis. J Pediatr Orthop. 39(2):90–97.

39. Montgomery CO, Siegel E, Blasier RD, Suva LJ. 2013. Concurrent septic arthritis and osteomyelitis in children. J Pediatr Orthop. 33(4):464–467.

40. Schlung JE, Bastrom TP, Roocroft JH, et al. 2018. Femoral neck aspiration aids in the diagnosis of osteomyelitis in children with septic hip. J Pediatr Orthop. 38(10):532–536.

41. Street M, Crawford H. 2015. Pediatric humeral osteomyelitis. J Pediatr Orthop. 35(6):628–633.

42. Ernat J, Riccio AI, Fitzpatrick K, et al. 2017. Osteomyelitis is commonly associated with septic arthritis of the shoulder in children. J Pediatr Orthop. 37(8):547–552.

43. Klenerman L. 2007. A history of osteomyelitis from the Journal of Bone and Joint Surgery: 1948 TO 2006. J Bone Joint Surg Br. 89(5):667–670.

44. Nickel JC, Ruseska I, Wright JB, Costerton JW. 1985. Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother. 27(4):619–624.

45. Lowenberg DW, Rupp M, Volker A. Understanding and treating chronic osteomyelitis. In: Browner B, Jupiter J, Krettek C, Anderson PA, eds. *Skeletal trauma.* 1. 6th. Philadelphia: Elsevier; 2020:707–742.

46. Zuluaga AF, Galvis W, Saldarriaga JG, et al. 2006. Etiologic diagnosis of chronic osteomyelitis: a prospective study. Arch Intern Med. 166(1):95–100.

47. Saiki RK, Gelfand DH, Stoffel S, et al. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 239(4839):487–491.

48. Kaplan HB, Miranda JA, Gogola GR, et al. 2018. Persistence of bacterial DNA in orthopedic infections. Diagn Microbiol Infect Dis. 91(2):136–140.

49. Yang S, Rothman RE. 2004. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis. 4(6):337–348.

50. Alt V, Lips KS, Henkenbehrens C, et al. 2011. A new animal model for implant-related infected non-unions after intramedullary fixation of the tibia in rats with fluorescent in situ hybridization of bacteria in bone infection. Bone. 48(5):1146–1153.

51. Dhiman N, Hall L, Wohlfiel SL, et al. 2011. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. J Clin Microbiol. 49(4):1614–1616.

52. Hirai J, Sakanashi D, Huh JY, et al. 2017. The first human clinical case of chronic osteomyelitis caused by Clostridium hydrogeniformans. Anaerobe. 45:138–141.

53. Melendez DP, Uhl JR, Greenwood-Quaintance KE, et al. 2014. Detection of prosthetic joint infection by use of PCR-electrospray ionization mass spectrometry applied to synovial fluid. J Clin Microbiol. 52(6):2202–2205.

54. Govaert GA, FF IJ, McNally M, et al. 2017. Accuracy of diagnostic imaging modalities for peripheral post-traumatic osteomyelitis – a systematic review of the recent literature. Eur J Nucl Med Mol Imaging. 44(8):1393–1407.

55. Al-Sheikh W, Sfakianakis GN, Mnaymneh W, et al. 1985. Subacute and chronic bone infections: diagnosis using In-111, Ga-67 and Tc-99m MDP bone scintigraphy, and radiography. Radiology. 155(2):501–506.
56. Chang N, Mathes SJ. 1982. Comparison of the effect of bacterial inoculation in musculocutaneous and random-pattern flaps. Plast Reconstr Surg. 70(1):1–10.
57. Calderon W, Chang N, Mathes SJ. 1986. Comparison of the effect of bacterial inoculation in musculocutaneous and fasciocutaneous flaps. Plast Reconstr Surg. 77(5):785–794.
58. Eshima I, Mathes SJ, Paty P. 1990. Comparison of the intracellular bacterial killing activity of leukocytes in musculocutaneous and random-pattern flaps. Plast Reconstr Surg. 86(3):541–547.
59. Gosain A, Chang N, Mathes S, et al. 1990. A study of the relationship between blood flow and bacterial inoculation in musculocutaneous and fasciocutaneous flaps. Plast Reconstr Surg. 86(6):1152–1162; discussion 1163.
60. Cox G, Jones E, McGonagle D, Giannoudis PV. 2011. Reamer-irrigator-aspirator indications and clinical results: a systematic review. Int Orthop. 35(7):951–956.
61. Bar-On E, Weigl DM, Bor N, et al. 2010. Chronic osteomyelitis in children: treatment by intramedullary reaming and antibiotic-impregnated cement rods. J Pediatr Orthop. 30(5):508–513.
62. Gauland C. 2011. Managing lower-extremity osteomyelitis locally with surgical debridement and synthetic calcium sulfate antibiotic tablets. Adv Skin Wound Care. 24(11):515–523.
63. Ferguson JY, Dudareva M, Riley ND, et al. 2014. The use of a biodegradable antibiotic-loaded calcium sulphate carrier containing tobramycin for the treatment of chronic osteomyelitis: a series of 195 cases. Bone Joint J. 96-b(6):829–836.
64. McKee MD, Wild LM, Schemitsch EH, Waddell JP. 2002. The use of an antibiotic-impregnated, osteoconductive, bioabsorbable bone substitute in the treatment of infected long bone defects: early results of a prospective trial. J Orthop Trauma. 16(9):622–627.
65. McKee MD, Li-Bland EA, Wild LM, Schemitsch EH. 2010. A prospective, randomized clinical trial comparing an antibiotic-impregnated bioabsorbable bone substitute with standard antibiotic-impregnated cement beads in the treatment of chronic osteomyelitis and infected nonunion. J Orthop Trauma. 24(8):483–490.
66. Henry SL, Ostermann PA, Seligson D. 1993. The antibiotic bead pouch technique. The management of severe compound fractures. Clin Orthop Relat Res. (295):54–62.
67. Morelli I, Drago L, George DA, et al. 2016. Masquelet technique: myth or reality? A systematic review and meta-analysis. Injury. 47 Suppl 6:S68-s76.
68. Blázquez J, Oliver A, Gómez-Gómez JM. 2002. Mutation and evolution of antibiotic resistance: antibiotics as promoters of antibiotic resistance? Curr Drug Targets. 3(4):345–349.
69. Baym M, Lieberman TD, Kelsic ED, et al. 2016. Spatiotemporal microbial evolution on antibiotic landscapes. Science 353(6304):1147–1151.

Chapter 8
Incidence, Complications and Novel Treatment Strategies: Joint Arthroplasty

A. Hamish R. W. Simpson

Abstract   There are ~1.5 million primary total joint arthroplasties (TJA) performed annually in North America, Australasia, and the United Kingdom. On average, the number of primary hip arthroplasty procedures increased by 30% between 2000 and 2015 and the number of primary knee arthroplasty procedures increased by almost 100%. The development of a prosthetic joint infection (PJI) has been shown to have major implications on patient-reported quality of life and function, healthcare costs, and risk of litigation. Cumulative treatment costs in the management of PJI in North America, Australasia, and the UK are estimated to be ~US$1.5 billion per annum. The ideal therapeutic goal in the management of PJI is generally accepted to be complete eradication of the pathogen and preservation of the joint function. This chapter reports on the contemporary incidence of PJI following knee, hip, ankle, shoulder and elbow arthroplasty as well as describes the general considerations and surgical strategies used to treat and manage PJI. This chapter also highlights the innovative approaches being developed to improve PJI on the organizational level as well as emerging treatment modalities targeted to inhibit transmission, bacterial adhesion, modulate metabolism, biofilm dispersion, novel antimicrobial agents, immunotherapy and methods designed to combat host intracellular penetration.

Keywords   Prosthetic joint infection · Biofilm · Treatment · Surgery · Knee · Hip · Shoulder · Elbow · Novel treatment strategies

8.1   Introduction

There are ~1.5 million primary total joint arthroplasties (TJA) performed annually in North America, Australasia and the UK [1–7]. Over 90% are total hip arthroplasties and total knee arthroplasties in approximately equal proportion [1–7]. In the most recent healthcare survey of countries participating in the Organisation for

A. Hamish R. W. Simpson (✉)
Department of Orthopaedic Surgery, University of Edinburgh, Edinburgh, UK
e-mail: hamish.simpson@ed.ac.uk

Economic Co-operation and Development, the number of primary hip and knee arthroplasties has increased rapidly since 2000. On average, the number of primary hip arthroplasty procedures increased by 30% between 2000 and 2015, and the number of primary knee arthroplasty procedures increased by almost 100% [8]. The annual number of joint arthroplasty procedures in North America, Australasia and the UK is expected to increase to nearly six million by 2030 [5, 9–11].

National surveillance programmes estimate the prevalence of prosthetic joint infections (PJI) to range between 0.2 and 5% [12–15]. A review of international registry data reported a global deep infection incidence 0.76–1.28% following TJA [16]. The incidence of PJI following primary total hip arthroplasty has not changed whereas with the average incidence of PJI following total knee arthroplasty has increased slightly from 0.88 to 1.03% [17]. Regardless of the low incidence of PJI, the soaring growth in the annual number of primary joint arthroplasty procedures means that the overall burden of PJI is rapidly increasing. The Nationwide Inpatient Sample in the USA reported that the absolute number of PJIs following total joint arthroplasty has more than doubled between 2001 and 2011 [18]. However, it should be noted that even national surveillance programmes have been shown to underestimate the true incidence of PJI due to under-reporting [19].

Gram-positive organisms account for the majority of PJI [15]. Coagulase-negative *Staphylococcus* (~40%), *Staphylococcus aureus* (*S. aureus*) (~20%), *Streptococcus* (~10%), *Enterococcus* (~5%), Gram-negative organisms (~5%) and anaerobes (~3%) account for the vast majority of monomicrobial infections [20]. In polymicrobial cases Gram-positive organisms are implicated in 70–80% of cases [15]. However, historic estimates of responsible pathogens are likely to be misleading, as some species, such as *Cutibacterium acnes* (*C. acnes)* (formerly *Propionibacterium acnes*), were previously considered to be non-pathogenic or 'weakly' pathogenic and often dismissed as contaminants. The true prevalence of *C. acnes* infection has been demonstrated through the application of more robust sampling and detection methods [21–23]. There has been greater acknowledgement of 'culture-negative' PJI, in which the clinical parameters of diagnostic criteria are fulfilled but no organisms can be isolated [24]. Prevalence of culture-negative PJI ranges from 5 to 41%, with 10% being the generally accepted estimate [25–27]. Reported reasons for negative cultures include fastidious organisms with demanding growth conditions, rare organisms not previously thought to be pathogenic or inadequate sampling [28]. However, the most important cause of culture-negative PJI is thought to be antibiotic administration prior to sampling [29, 30]. Sub-therapeutic antimicrobial therapy is known to induce a physiological state in many pathogens known as 'viable but non-culturable' [31–34], rendering cultures falsely negative [30, 35]. This is a cellular state characterised by low metabolic activity and failure to grow on routine bacteriological media [36]. A critical feature is that nutritional stimulation can restore metabolic activity and culturability, known as resuscitation [37, 38].

The development of PJI has been shown to have major implications on patient-reported quality of life and function [39–41], healthcare costs [12, 42] and risk of litigation [39]. They are associated with a 2–4% 90-day mortality [43, 44], rising to

a 20–26% 5-year mortality [44, 45]. The 5-year mortality of PJI has been reported to be greater than four of the five most commonly diagnosed cancers in the USA [46]. According to economic estimates in the USA, the total hospital treatment costs for an individual patient with PJI range from US$30,000 to 120,000 [47–49], with similar estimates reported for the UK [12, 50]. Cumulative treatment costs in the management of PJI in North America, Australasia and the UK are estimated to be ~US$1.5 billion per annum [9, 12, 51, 52].

8.2 Treatment

8.2.1 General Considerations

There are several management strategies for PJI, the choice of which is guided by an understanding of the pathogen, host and the local site of infection. The importance of these factors has been highlighted in the PJI staging system proposed by McPherson et al. [53] (Table 8.1) and the treatment algorithm from Zimmerli et al. [54] (Figs. 8.1 and 8.2). These have been adopted widely in clinical practice, which is thought to be a key driving force behind the improving outcomes of PJI in recent years [28, 55]. These systems and algorithms also place importance on the chronicity of infection, with a threshold of under 4 weeks since onset of symptoms deemed to be critical in outcome. However, a recent systematic review and meta-analysis of cohort studies has reported that there is a more favourable outcome when debridement and implant retention procedures are performed within 7 days of symptom onset [55]. Despite these studies there has been a recent movement to re-examine the traditional temporal classification of infection [56]. It is thought that the apparent success with lower morbidity strategies seen with early infections may, in fact, be a reflection of related host and pathogen factors rather than the application of a binary decision-making process driven by 'time from symptom onset'. It has been proposed that the chronicity of infection viewed as a continuum (early to late/established) within a wider framework of host, pathogen and circumstantial factors would be more reflective of the current understanding in the pathophysiology of PJI [56].

The overall goal of treatment is the eradication of infection and preservation of patient function. A recent international consensus meeting has stratified the eradication of infection, moving away from the traditional dichotomous classification [57]. Functional outcomes have been reported using general health and joint-specific patient-reported questionnaires such as the EQ-5D and Oxford hip score [58–61], respectively, as well as qualitative methodologies [62].

Current attempts to combat biofilm infections are largely based on early and aggressive physical removal (debridement and irrigation +/− excision) with the administration of local [63] and systemic antimicrobial therapy [64]. Generally, developments have been limited to the modifications of systemic and local

Table 8.1 McPherson staging system

Category	Grade	Description
Infection	I	Prosthetic joint infection (PJI) < 4 weeks after implantation
	II	Acute haematogenous PJI < 4 weeks duration of symptoms
	III	Late and chronic PJI > 4 weeks duration of symptoms
Host (systemic)	A	No compromising factors[a]
	B	Compromised (≤ 2 factors)
	C	Significant compromise (> 2 factors) or one of the following:
		Neutrophil count <1000 cells/mm^3
		CD4+ T-cell count < 100 cells/mm^3
		Intravenous drug abuse
		Chronic active infection (distant to joint)
		Dysplasia/neoplasm of immune system
Local site	1	No compromising factors[b]
	2	Compromised (≤ 2 factors)
	3	Significant compromise (> 2 factors)

[a]Systemic compromising factors include age > 80 years, alcoholism, chronic active dermatitis or cellulitis, chronic indwelling catheter, chronic malnutrition (albumin < 3.0 g/dl), current nicotine use (inhalation or oral), diabetes mellitus (non-diet controlled), hepatic insufficiency (cirrhosis), immunosuppressive medications, malignancy (history of or active), pulmonary insufficiency (arterial saturation ≤ 60% on room air), renal dialysis, systemic inflammatory disease (rheumatoid arthritis, systemic lupus erythematosus) and systemic immune compromise from infection or disease (e.g. HIV or AIDS)

[b]Limb compromising factors include local active infection present > 3 months, multiple previous incisions creating skin bridges, soft tissue loss from prior trauma, subcutaneous abscess > 8 cm^2, synovial cutaneous fistula, prior periarticular fracture or trauma (especially crush injury), prior local irradiation to wound area and vascular insufficiency (absent limb pulses, chronic venous stasis disease, significant calcific arterial disease).

antibiotic protocols [65–67] and revisiting formerly 'last-resort' antibiotics such as colistin [68]. The key component of current management is physical removal of the biofilm [69]. Debridement has been a central tenet in the management of musculoskeletal infections since the early twentieth century. The etymology of 'debridement' derives from the French *débrider* meaning to unbridle, as the term originally referred to deliberate wound extension and fragment removal as described by Ambrose Paré in the sixteenth century [70]. The current use of the word debridement refers to the excision of all devitalised and (macroscopically) contaminated tissue [71]. Debridement can be viewed as either superficial or deep. Superficial wound debridement can be further subcategorised: (1) autolytic, through the use of hydrogels and auto-enzymes; (2) enzymatic, using streptokinase and collagenase; and (3) biological, the most widely used being maggot therapy [72]. Deep wound debridement can also be subcategorised [69]: (1) surgical, which includes soft tissue dissection and excision +/− prosthesis explantation; (2) mechanical (e.g. bone curettage and reaming, power lavage and H_2O_2 [73]); and (3) chemical, which can
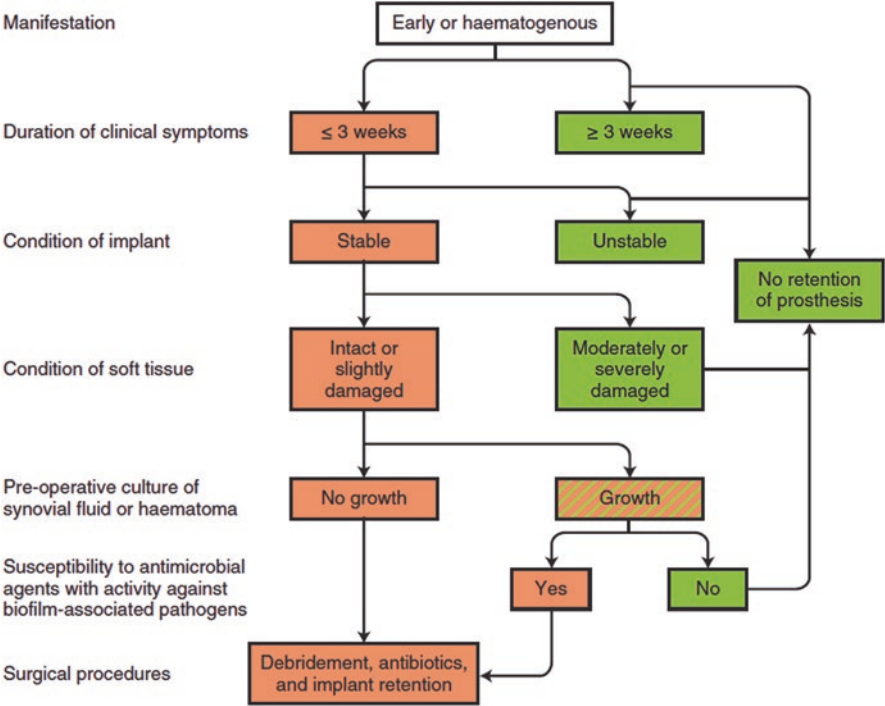
Fig. 8.1 Treatment algorithm for early and haematogenous prosthetic joint infections by Zimmerli et al. [54]
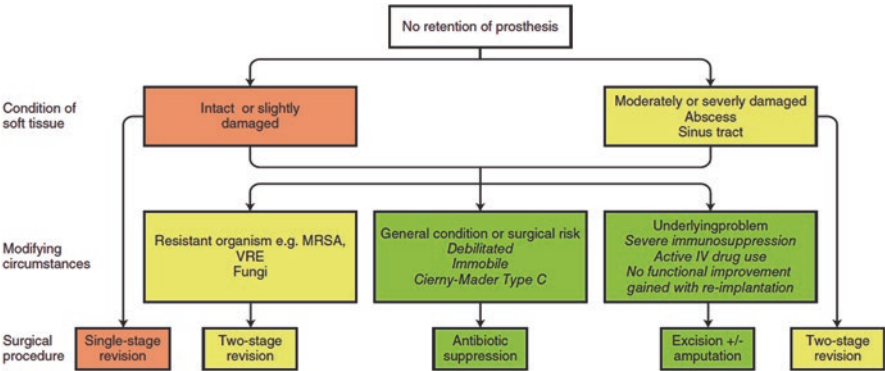
Fig. 8.2 Treatment algorithm for prosthetic joint infections not eligible for implant retention by Zimmerli *et al.* [54]

include acetic acid [74, 75] and honey [76]. Given more recent developments in the understanding of the pathogenesis of PJI, debridement of intracellular pathogens [77, 78] should also be targeted [77–80]. A further advantage of open debridement is the opportunity to administer local antimicrobial therapy. The enhanced local soft tissue concentration associated with use of antimicrobial depots assists in the eradication the residual biofilm and prevents recolonisation. A local antimicrobial depot does not rely on the presence of vascularised tissues and achieves concentrations that are orders of magnitude higher than can be achieved safely using systemic administration. The local application of antibiotics in orthopaedic medicine was first described in the 1970s, when gentamicin-loaded bone cement was first tested in humans [63]. Bone cement was a convenient vehicle for antibiotic delivery, as it was routinely applied in cemented arthroplasties. Gentamicin was identified as a suitable antibiotic due to the fact that it was found to withstand the heat energy produced during the exothermic reaction involved in the curing process, as well as providing an acceptable susceptibility profile against the most common pathogens associated with PJI. There is evidence to show that bone cement is effective in minimising the risk of PJI following primary hip and knee arthroplasty [81–83]. The use of locally delivered antibiotics in the management of PJI has been shown to be associated with effective eradication in 75–91% cases [54, 84]. Bone cement, however, was not primarily designed as an antibiotic delivery vehicle. Therefore, the usual pharmacodynamic principles which govern systemic antibiotic doses were not considered during the introduction of antibiotic-loaded bone cements. Unfortunately, despite the passage of more than four decades since the first use of antibiotic-loaded bone cements, optimal dosing has yet to be established for use in this role. Therefore, it is perhaps not surprising that reports have emerged of resistance against gentamicin when used in local delivery vehicles [85, 86]. The reason for the development of resistance is thought to be due to the prolonged release of antibiotics at subtherapeutic levels from local delivery vehicles, which is in direct opposition to ideal release kinetics for a concentration-dependent antibiotic such as gentamicin [87]. There are antimicrobial-loaded device surfaces and coatings (e.g. antibiotic-coated nails and silver-coated endoprostheses), and biodegradable systems (e.g. collagen fleeces, calcium sulphate pellets and hydrogels) which have obtained regulatory approval and have shown potential in clinical studies [88–91]. Despite the availability of alternative systems, antibiotic-loaded bone cement remains the most commonly used antibiotic carrier in the management of orthopaedic device-related infections, including PJI [92, 93]. A further tenet of current management is the use of systemic antimicrobials. These are typically broad-spectrum agents with good connective tissue penetration and delivered intravenously in the initial postoperative period, with rationalisation to pathogen-directed agents when isolates become available. Traditional dogma states that antimicrobials should be administered intravenously for a period of 2–6 weeks [94] in the postoperative period. However, there is growing evidence to suggest that shortening the duration of intravenous therapy does not result in inferior outcomes [95–97]. Previously it was felt that the evidence for this claim was weak due to the confounding factors and inherent biases within these non-randomised studies [98]. A recent randomised controlled trial found

non-inferiority in treatment failure at 1 year when 6 weeks of oral antimicrobials were compared with 6 weeks of intravenous agents in bone and joint infections [99]. Although it should be noted that 35% of patients enrolled did not have surgical implants or prostheses present at the site of infection [99]. A further consideration should be the interactions of antimicrobial agents that are being administered in combination. Recent in vitro biofilm studies have demonstrated unexpected synergistic and antagonistic effects of antimicrobial combinations commonly used in staphylococcal PJI [65, 100]. These interactions have not been previously observed when using standard laboratory susceptibility testing models [101].

8.3   Current Surgical Strategies

The ideal therapeutic goal in the management of PJI is generally accepted to be complete eradication of the pathogen and preservation of the joint function. Current surgical management strategies can be broadly classified along the lines of prosthesis retention and associated patient morbidity (Fig. 8.3). However, the choice of treatment for PJI generally depends on a number of factors, including local factors referring to the bone and tissue condition, fixation and stability of the prosthesis, the chronicity of infection, the type of organism and the host's condition [54, 102–104]. For patients with multiple comorbidities, it may be appropriate to avoid surgical intervention and to pursue antibiotic suppression. In the case of early-onset infections, debridement, antibiotics and implant retention (DAIR) can be a first-line option [54, 102]. Revisions are an alternative treatment to DAIR and can be performed as single- or two-stage procedures [105]. In single-stage revisions, the
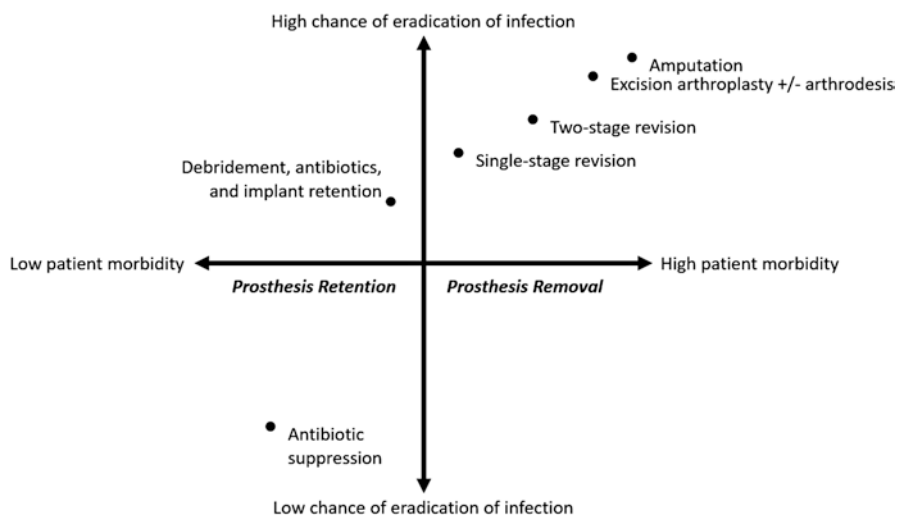
Fig. 8.3 Current management strategies in periprosthetic joint infection

prosthesis is exchanged during the same surgery as the debridement. Two-stage revisions, on the other hand, involve removing the prosthesis and treating the patient with systemic antibiotic therapy (typically 6 to 8 weeks), before inserting a new prosthesis once the infection is controlled [106]. Salvage procedures include removal of the prosthesis permanently (excision arthroplasty) +/- arthrodesis and limb amputation [107].

8.3.1 Antibiotic Suppression

Antibiotic suppression is generally recommended only in (1) patients whose medical comorbidities preclude them from surgical intervention (i.e. the 'type C' host as per the Cierny-Mader staging system for osteomyelitis [108]), (2) patients who have experienced multiple failed surgical interventions and further surgery is unlikely to improve functional outcome and (3) patients who refuse surgery [109]. Ideally, the pathogen should be of low virulence and sensitive to an orally administered antimicrobial agent, there should be no evidence of sepsis and the prosthesis remains well fixed [110]. Successful eradication of infection with antibiotic suppression only is estimated to be ~ 20% [111]. The true objective of this strategy is not curative but instead to suppress bacterial replication and dissemination to minimise symptoms and systemic upset. Compliance with lifelong antimicrobials can be difficult for some patients to manage with adverse effects being commonly reported [28]. However, the lack of robust evidence evaluating the efficacy of antibiotic suppression in the management of PJI has been widely acknowledged [109, 112].

8.3.2 Debridement, Antibiotics and Implant Retention (DAIR)

DAIR is considered to be a low morbidity treatment strategy. It allows the primary prosthesis to be retained through a single surgery, limiting patient morbidity and functional impairments associated with implant excision [60, 113]. Prosthesis removal can lead to bone loss and soft tissue destruction, limiting reconstruction options. DAIR can be performed as an open or arthroscopic procedure. The basic principles of DAIR are to perform an open arthrotomy, explant all exchangeable components (to optimise access and visualisation) while leaving the fixed components in situ, obtain multiple fluid and tissue samples for microbiology and histological investigation, excise all necrotic and/or infected soft tissue, irrigate the joint with large volumes of fluid and finally replace explanted modular components. Exchanged components are typically the polyethylene liner for knee replacements and the femoral head and acetabular liner for hip replacements [114]. Exchange of modular components has been shown to improve the likelihood of infection being eradicated [55]. Arthroscopic DAIR is theoretically associated with even lower morbidity, but several limitations have been identified, namely, an inability to assess

prosthesis stability and to exchange modular components, as well as a lack of access to perform a complete circumferential debridement of joint space and synovium [110]: it is, therefore, generally not recommended.

8.3.3 One- or Two-Stage Exchange

A single-stage exchange (and DAIR) offers certain advantages over two-stage procedures such as the need for only one operative procedure (if there is no recurrence of infection), reduced time of in-patient care and treatment costs and improved functional and patient-reported outcomes [60, 62, 104, 115–118]. Classically DAIR and single-stage revision was reserved for acute infections ($\leq$ 3 weeks), when the pathogen and its sensitivities had been identified [54] and with no evidence of septic loosening of the prosthesis (in the case of DAIR). There has been a shift away from considering temporal factors in the decision-making process. A recent international consensus meeting on PJI concluded with 94% agreement amongst invited delegates that cases of PJI where symptoms had been present > 2–4 weeks did not mandate two-stage revision, provided the other indications for DAIR or single-stage revision were met [119]. Single-stage revisions and DAIR were associated with better functional and patient-reported outcomes but historically have reported lower eradication success [105, 120]. There is a very wide range of reported proportions of success for DAIR in the management PJI ranging from 14% [121] to 100% [122–124]. However more recent cohort studies and a systematic review of cohort studies have demonstrated more favourable success with DAIR [55, 60, 125] and single-stage revisions [104]. Contraindications to one-stage revision include (1) the presence of antibiotic-resistant pathogens, (2) the presence of a sinus tract or (3) compromised soft tissue coverage. The presence of one or more of these features necessitates a two-stage revision strategy, which has been described by some to be the gold standard for PJI management [126, 127]. Series following this treatment rationale have reported infection eradication success > 90% [43, 54, 128, 129]. Given that better functional and patient-reported outcomes are associated with DAIR and single revision, there is a clinical need to optimise the lower morbidity treatment options.

8.3.4 Salvage

Salvage procedures such as resection arthroplasty +/− joint arthrodesis and limb amputation should only be considered after all other alternatives have been exhausted. Resection arthroplasty involves the surgical debridement and removal of all prosthetic material including acrylic bone cement from the bed of infection. It offers the advantages of a limited surgical time and removal of foreign material from the focus of infection, but at the cost of joint function [130, 131]. Joint

arthrodesis is generally reserved for knee PJI, with limited application in the context of prosthetic hip joint infection [131]. Knee arthrodesis may be accomplished in a variety of ways: (1) intramedullary fixation (modular and non-modular), (2) external fixator (uni-/biplanar, hybrid and fine-wire frames) and (3) plating techniques [130]. No single technique has been shown to be superior to others regarding fusion rates, complications or postoperative function [130]. Limb amputation is a last-resort option for patient with infected ankle and knee prosthesis who have exhausted all treatment options but are not candidates for resection or arthrodesis, with removal of the lower extremity at the level of the tibia (below knee amputation) or femur (above knee amputation). Amputation may be indicated in patients who have had multiple procedures with failure to eradicate infection resulting in bone loss, incompetent soft tissue stabilisers and inadequate soft tissue coverage [132]. It is thought that the final surgical, functional and patient-reported outcomes of salvage procedures can be improved with thorough preoperative counselling that explores the associated functional limitations, demands and expectations of the patient [132].

8.4   Knee Arthroplasty

The infection burden following primary TKA, estimated from international arthroplasty registries, is 1.03% (range, 0.88–1.28%) [16]. The burden of infection is even greater following revision TKA; for all-cause revision (including infection), it is estimated to be 8.25% [133]; for cases of aseptic revision only, it is estimated to be 2.1% [134]. Data extracted from the Nationwide Inpatient Sample in the USA demonstrated that the absolute number knee PJI increased from 7113 to 16798 between 2001 and 2010, which represented 2.05% and 2.32% of annual primary total knee replacements performed, respectively [18]. PJI is the second most frequent complication following primary TKA (behind aseptic loosening) [135], with the average cost of revision in the USA estimated to be $49,360 and length of in-patient stay of 5 days, representing a significant burden to healthcare institutions [48] and, more importantly, to physical and psychological well-being of patients and their families [62, 136]. However, up to 10% of failed TKA due to aseptic loosening are thought to represent clinically occult PJI [137].

Historically, the reported success with DAIR in the treatment of acute knee PJI was ~ 50% [138], with rates as low as 30% in some larger series [139, 140]. A recent systematic review of the literature reported > 65% overall success (range, 16–100%) in infection eradication following DAIR in acute TKA PJI [141]. The authors identified acute PJI (early postoperative and haematogenous), non-resistant pathogens, selection of appropriate antibiotics targeting the high virulence organisms and exchange of the modular components to be critical prognostic factors in their review [141]. It was concluded that in highly selected series of patients, DAIR for TKA PJI can have comparable success to those undergoing exchange procedures [141]. A further recommendation suggested by an international consensus meeting was the importance of performing a synovectomy during debridement in TKA PJI [119].

Following initial failure of DAIR, further repeated attempts have been shown to have limited success and should be avoided [142].

In addition to the general indications, described by Zimmerli et al. [54], for single-stage revision is the necessity for preoperative identification of causative pathogen and an antibiogram that facilitates local antibiotic delivery. The implantation of locally delivered pathogen-directed antibiotics prevents colonisation and biofilm formation of the newly implanted prosthesis [143]. The institute that originally described the technique (using antibiotic-loaded bone cement) reports 75–80% infection eradication when single-stage revision is used to treat TKA PJI [144]. A suggested technical point by Haasper and Gehrke is the importance of an aggressive approach to debridement with a mandatory total synovectomy (including the posterior knee capsule) and at least a consideration to resect ligamentous structures. They go on to recommend that joint stability should be subsequently regained using an appropriately constrained implant such as a hinged prosthesis [143]. Although this advice appears appropriate for the purposes of infection eradication, the evidence for this claim is not robust.

Two-staged revision is considered by some to be the gold standard treatment for PJI of the knee [145] with eradication rates between 88 and 100% [127, 146–150]. Following debridement and explantation of the infected joint, a local antibiotic depot is implanted to prevent recolonisation of the joint space, preserve bone stock, maintain soft tissue tension and prevent joint contractures [145]. Early spacers did not articulate [151, 152], which led to quadriceps contracture, arthrofibrosis, extensor mechanism disruption, spacer migration and bone loss [146]. Articulating spacers, popularised almost 25 years ago [153], were developed to overcome these issues, resulting in greater patient comfort during the interim period and a technically easier reimplantation for the surgeon [146, 154, 155]. In the intervening years a number of different articulating spacers have been developed: cement-on-cement, cement-on- polyethylene and metal-on-polyethylene articulations [145]. The use of an articulating spacers in the management of PJI following TKA is dependent on the presence of adequate bone stock, an intact extensor mechanism and an adequate soft tissue envelope, with the loss of the extensor mechanism thought to be an absolute contraindication [145].

The current role for knee arthrodesis in relation to TKA PJI remains a moot point, but an accepted indication is for the patient with an unsalvageable infected TKA on the background of recurrent infection, often following multiple revisions, in order to prevent progression to an above knee amputation [156]. Factors that are generally considered to favour arthrodesis over further reconstruction include the presence of multi-resistant pathogens, gross instability and a compromised soft tissue envelope (including extensor mechanism deficiency and functionally limiting joint stiffness) [156]. The only absolute contraindication to arthrodesis would be in the case of life-threatening sepsis from PJI of the knee where amputation may be the only option [156]. Relative contraindications include patients with a contralateral amputation, and knee arthrodesis may be unsuitable due to the degree of energy expenditure during walking. Knee arthrodesis requires the exertion of 30% more energy compared to a normal gait, with amputation 25% greater than arthrodesis

[157], making knee arthrodesis also unsuitable in patients with limited cardiorespiratory reserve [156]. Arthrodesis can be achieved using internal fixation (intramedullary [158] and extramedullary [159]), external fixation [160] and vascularised strut grafts [161]. Above-knee amputations in the context of knee PJI is a last-resort treatment option, for those that have exhausted all other treatment strategies and are unsuitable for further two-stage revision and/or arthrodesis. Following amputation patients should be counselled about the risk of wound dehiscence, skin necrosis, bone erosion, heterotrophic ossification, haematoma, oedema, nociceptive pain and neuropathic pain (e.g. neuromas and phantom limb syndrome). Generally patient-reported outcomes following amputation secondary to knee PJI are poor, primarily due to the increased energy costs with ambulation [162], resulting in reduced walking speed and increased oxygen consumption [163]. The final surgical outcome and satisfaction following salvage procedures, such as knee arthrodesis or above-knee amputation, may be improved through preoperative discussion with the patient regarding functional limitations, demands and expectations in a multidisciplinary team environment [130–132].

8.5 Hip Arthroplasty

The infection burden following primary THA, estimated from international arthroplasty registries, is 0.97% (range, 0.76–1.24%) THA [16]. The burden of infection is even greater following revision THA; following revision for aseptic causes, there is an estimated 1.6% risk of subsequent PJI [164]. Data extracted from the Nationwide Inpatient Sample in the USA demonstrated that the absolute number of PJIs following THA grew from 4545 to 8858 between 2001 and 2011 [18]. The average total cost of treatment for PJI following THA in the USA has been estimated to be $93,600, with the average length of in-patient stay ~10 days [49].

Treatment of acute THA infections with DAIR was first reported in 1974 by Müller [165] and then by Coventry [166] in 1975 with 80% and 20% infection eradication achieved, respectively. Burton and Schurman [167] reported their experience with the technique in 1977 with 75% of patients remaining infection-free at follow-up. The approach by Burton and Schurman was 'radical debridement of all necrotic debris and removal of the prosthesis, where the prosthetic components were loose, or bone involvement was present. The patients were treated with wound irrigation with an appropriate antibiotic and were maintained on high doses of parenteral antibiotics for as long as possible' [167]. Muller and Coventry described similar techniques with Coventry advocating 'closure over tubes' to encourage drainage of purulent material [166]. With further reports of experience with the technique, factors thought to improve treatment success included onset of PJI within the first 4 weeks following implantation [168], debridement initiated early after the onset of symptoms of infection [169], the absence of a sinus tract or radiographic signs of implant loosening at the time of debridement [170] and the type, duration and route of antimicrobial therapy [171–174]. The absolute contraindications for

DAIR include (1) the presence of a loose prosthesis, (2) poor soft tissue coverage and (3) bone cement mantle compromise [114]. Historically there has been a very wide range of reported proportions of PJI eradication following DAIR, ranging from 14% [121] to 100% [122–124]. A recent systematic review and pooled analysis of case series found that outcomes had improved since 2004 and the publication of the treatment algorithm by Zimmerli et al. [54], successful eradication being achieved in 72% of published cases [55]. Further improvement in outcomes was seen with debridement undertaken within 7 days on symptom onset and the exchange of modular components [55]. Patient-reported and functional outcomes following a single successful DAIR have been shown to be comparable to those of age- and sex-matched patients having undergone primary THA. In the same study DAIR was also found to have comparable rates of infection eradication to matched patients undergoing two-stage revision for hip PJI but with superior Oxford hip scores [60].

There is currently equipoise between revision strategies for cases of hip PJI where prosthesis retention is precluded [175]. Surgical revision for a hip PJI involves prosthesis removal, debridement, antibiotic treatment and re-implantation of a new prosthesis. The prosthesis is replaced in the same operation (single-stage) or replaced at a delayed interval (two-stage), ranging from 2 weeks to 12 months. In a two-stage revision, a temporary 'spacer' or temporary joint replacement may be fitted, but the patient has no definitive prosthesis until it is replaced in the second operation. Two-stage revision has the potential for additional antimicrobial therapy, through the use of an antibiotic-eluting spacer, but at the expense of patient function and quality of life [176]. Single-stage revision is becoming increasingly popular as they are thought to be associated with superior functional outcomes [176], a more acceptable patient experience [136] and reduced healthcare costs [177]. Historically two-stage revision was associated with superior infection eradication rates (> 90%) [54] and was considered to be the treatment 'gold standard', particularly in North America [178]. However, a systematic review and meta-analysis of the literature found that there were now similar rates of eradication success between single-stage and two-stage strategies (91.8% vs 92.1%) [179]. It is hoped that an ongoing randomised superiority trial in the UK, INFection ORthopaedic Management (INFORM), will provide evidence to break this equipoise [175].

Salvage options in hip PJI are resection arthroplasty and hip disarticulation. Generally, resection is indicated in 'C-type' hosts [108], those who refuse further surgery and in cases of severe bone loss and/or soft tissue compromise [180]. A cohort study of patients undergoing resection arthroplasty following hip PJI reported that 85% patients experienced either a minor or major complication, 42% requiring a secondary procedure and 50% mortality at ~ 4 years [181]. Hip disarticulation is typically reserved for patients who have had numerous failed attempts at revision or those with life-threatening soft tissue infections associated with their hip PJI [180]. There is a spectrum of outcomes reported in the literature following hip disarticulation, ranging from successful prosthetic rehabilitation [182] to 63% affected by postoperative wound infection and 44% mortality [183]. However, it should be noted that these outcomes are based on small heterogenous case series (< 50).

8.6   Ankle Arthroplasty

Ankle arthroplasties represented only 0.3% joint replacements recorded in the UK National Joint Registry in 2017 [6]; however, they have been found to be a higher risk of PJI compared with hip and knee arthroplasties (up to 13%) [184, 185]. Risk factors identified for ankle PJI include low body mass index, inflammatory arthritis, peripheral vascular disease and diabetes mellitus [186]. The literature reporting on the outcomes of ankle PJI is limited to small case series. A systematic review and pooled analysis of outcomes following surgical treatment of ankle PJI reported that DAIR successfully eradicated infection in 14/27 (52%) cases and in 57/72 (79%) cases undergoing revision arthroplasty [187]. Arthrodesis was reported to eradicate infection in 29/30 (97%) cases, 24/30 going on to successful fusion but of which only 12/30 obtained a 'good' functional outcome with a stable plantigrade foot with minimal or no limp [187]. A permanent antibiotic-eluting cement spacer, used in cases with tissue loss, recalcitrant infection, or 'type C' hosts, was complicated in 4/12 cases (subluxation ($n = 3$) and symptomatic loosening ($n = 1$)). Amputation used as a primary treatment led to 9/9 (100%) cases remaining free from infection. Due to the paucity of available evidence, there is currently no universally agreed treatment algorithm for the management of ankle

8.7   Shoulder Arthroplasty

The combined mean incidence of shoulder arthroplasty procedures in a review of national registries in the USA, Australasia and Europe was a 20/100000 with 2.6-fold in the proceeding decade [188]. A sixfold variation of incidence was reported between the highest (Germany) and lowest (UK) countries [188]. In the UK, shoulder arthroplasty represented only 3.1% (95% confidence intervals (CI) 3.06–3.20) of all joint arthroplasties in 2017 [6], with 0.3% (95% CI 0.27–0.41) shoulder arthroplasties revised for infection [6]. In a systematic review of shoulder arthroplasty by Bohsali et al., it was reported that PJI complicated 0.7% of all procedures and that PJI made up 4.6% of all complications [189]. An analysis of the Nationwide Inpatient Sample in the USA between 2002 and 2011 reported a shoulder PJI prevalence of 0.98% [190] following primary procedures, with the prevalence following revision shoulder arthroplasty estimated to be between 4 and 15% [190, 191]. The estimated median cost of total care for shoulder PJI is $17,164 [190].

A scenario pertinent to shoulder arthroplasty is the relatively high prevalence of unexpected positive cultures following supposedly aseptic revisions. Unexpected positive cultures have been reported to be present in 15–29% revision cases where PJI was not clinically suspected [192–194]. In published case series, *C. acnes* was isolated in 57–83% of cases [193–195]. A pooled analysis of 1405 aseptic revision reverse shoulder arthroplasties estimated the prevalence of unexpected positive cultures to be 17%, with *C. acnes* isolated in 63% of these cases [196]. *C. acnes* is an

anaerobic, Gram-positive bacillus that preferentially colonizes the shoulder compared with the knee and hip joints [197, 198]. *C. acnes* was previously thought to be non-pathogenic, but it has been shown to be capable of forming biofilms [199] and is subsequently pathogenic [192, 200]. Because of the fastidious nature of *C. acnes*, prolonged culture time (2–4 weeks) is often required to isolate the organism; however, incubation times beyond 2 weeks increase the likelihood of contamination and therefore false-positive results [201]. It is unclear whether unexpected *C. acnes* cultures represent true infection, inoculation of the deep tissues by skin commensals or laboratory contamination [202]. Using reoperation as the outcome measure for confirmed infection, two studies have reported that 1/28 (4%) [195] and 2/8 (25%) [193] unexpected positive cultures were true infections. An investigation to estimate the incidence of *C. acnes* colonisation in open shoulder surgery found that 24/117 (21%) cases had at least one sample that was culture positive. However, in the same study 7/54 (13%) sterile swabs, sent as controls alongside the intraoperative pericapsular samples, were also culture positive [194], suggesting that a large proportion of the unexpected positive cultures are due to contamination. Subsequently, an international consensus meeting recommended against mandatory therapeutic antibiotic therapy in cases of revision surgery that yield unexpected positive cultures of low virulence organisms, such as *C. acnes* [203].

The literature reporting the outcome following DAIR in both acute and subacute/chronic cases of shoulder PJI is limited. For acute infections a pooled analysis of published case series (38 shoulder arthroplasties in 37 patients) found that only 19/38 (50%) shoulders were infection-free at follow-up following DAIR [203]. In a pooled analysis of 51 cases of subacute/chronic case, DAIR eradicated PJI in only 24/57 (47%) cases. Stone et al. described a case series of 79 patients with shoulder PJI treated with debridement and partial component exchange (*n* = 15) compared with patients with single-stage revision (*n* = 45) and two-stage revisions (*n* = 19). Single-stage revisions were found to eradicate infection in 43/45 (96%) cases compared with only 11/15 (63%) eradication following debridement and partial exchange of components [204]. The study concluded that although there may be some circumstances in which retaining a prosthesis is preferable (e.g. well-fixed non-modular components), surgeons should be aware of the reduced likelihood of infection eradication [204]. A retrospective multicentre study from France described 32 patients who underwent surgical treatment for infection after reverse shoulder arthroplasty. Within this cohort 13 patients underwent debridement, modular component exchange and partial component retention, with only 7/13 (54%) patients successfully cleared of infection at follow-up. However, the 15% complication rate reported with debridement was lower than that reported for resection (33%), single-stage revision (20%) or two-stage revision (36%). In addition, those treated successfully with DAIR were also found to have superior Constant shoulder scores [205].

Once again, the literature examining the indications and outcomes of single-stage and two-stage revision in shoulder PJI is limited. A review of the literature identified 12 retrospective case series involving 161 patients undergoing single-stage revision and 27 retrospective case series with 325 patient undergoing two-stage revision [203]. Single-stage revisions were found to have a higher likelihood

of infection eradication (94.4% vs 88.6%), comparable functional outcomes and lower risk of complication (12.7% vs 21.9%). However, it should be noted that these retrospective studies are at risk of both selection and reporting bias with no published comparative studies yet available.

A further consideration in the revision of infected shoulder arthroplasties is component selection. Conversion to a reverse polarity shoulder arthroplasty may be preferred to an anatomic implant where there is evidence of rotator cuff incompetence and/or bone loss in the proximal humerus or glenoid following debridement [191, 206, 207]. Infection and soft tissue loss have been found to be associated with poor functional outcomes after aseptic revision, with anatomic prostheses compromised further by rotator cuff dysfunction and instability [207–209]. Reverse polarity prostheses have been reported to better compensate for soft tissue loss or bone deficiency [207, 210], improving improve pain control and overall functional recovery without a compromise in infection eradication [210–214]. In cases of shoulder PJI with an intact rotator cuff, revision to hemiarthroplasty is considered by some to be reasonable option with comparable results to reverse polarity implants in the revision for PJI [212, 215, 216]. Other scenarios where conversion to a hemiarthroplasty rather than reverse polarity prosthesis is preferable include cases of substantial glenoid bone loss, recurrent instability of a previous reverse polarity prosthesis and patient factors such as non-compliance precluding implantation of a reverse arthroplasty implant [217, 218]. Although better pain relief and functional scores can be obtained with anatomic shoulder arthroplasties compared with hemiarthroplasty in revision surgery [219], the rate of polyethylene glenoid component loosening is clinically significant [220]. In the context of shoulder PJI, conversion to an anatomic prosthesis should be strictly limited to cases in which the rotator cuff is intact and fully functioning, glenoid bone stock is sufficient and bacterial burden is minimal [203].

8.8   Elbow Arthroplasty

Elbow arthroplasties represented 1.4% (95% CI 1.33–1.43) of all joint arthroplasties recorded in the UK National Joint Registry in 2017 [6]. The incidence of total elbow arthroplasty (TEA) grew 248% from 1993 to 2007, and the incidence of upper extremity revision arthroplasty grew 500% during the same time period [221]. This rise in surgical volume has led to a similar rise in volume of surgical complications, including infection. The risk of infection after TEA is substantially higher than the risk after shoulder arthroplasty or even hip and knee arthroplasty. Earlier studies reported risk of deep infection at approximately 10%, though larger more recent cohorts estimate it to be closer to 3% [222, 223].

PJI reported as a cause for revision within the UK National Joint Registry is 1.13% for elbow arthroplasty [224]. The higher risk of infection in elbow arthroplasty as compared to hip, knee and shoulder prostheses is thought to be due to a number of confounding factors: (1) the main indication for hip or knee replacement

is degenerative osteoarthritis; in primary elbow arthroplasty rheumatoid arthritis and post-traumatic osteoarthritis are the most common indications (autoimmune inflammatory arthritides are known to confer a higher risk of infection due to the presence of a chronic inflammatory state and the use of immunomodulatory disease-modifying treatments to manage these conditions [225]); (2) the minimal soft tissue envelope around the elbow provides limited barrier protection against contiguous spread of infection following superficial infections (e.g. bursitis and superficial wound infections); and (3) the soft tissue envelope is more vulnerable and less effective as a barrier to infection in patients with post-traumatic or inflammatory arthritis due to traumatically damaged tissue, previous surgery or skin atrophy secondary to corticosteroid use [222]. This is evidenced with the higher prevalence of infection in patients with either rheumatoid arthritis (~ 5%) [226, 227] or in the revision setting (8%) [228].

The Yamaguchi classification system is a commonly used to aid decision-making in the management of infected elbow arthroplasties [223]: Group I infection with stable implant, Group II infection with unstable implants and adequate bone stock and Group III infection with poor stock that prevents reimplantation. According to a recent consensus guideline from the British Elbow and Shoulder Society, DAIR should only be considered in patients with a Yamaguchi type 1 infected elbow arthroplasty that has been in situ for ≤ 3 months, has had a duration of symptoms of < 3 weeks, has adequate soft tissue cover and the pathogen isolated preoperatively known to be sensitive to antibiotics active against biofilms [229]. The indications for DAIR detailed by the British Elbow and Shoulder Society were based on the risk factors identified in a cohort study of 27 infected elbow prostheses [230]. Two-stage revision should be considered in Yamaguchi type I infections that do not fulfil the criteria for DAIR or those with type II infections [229]. Resection arthroplasty should be reserved in patients with Yamaguchi type III infections. Improved functional outcomes are thought to be associated following resection when both medial and lateral columns of the humerus are preserved [229]. Delayed reconstruction with bone allograft to allow reimplantation is an option, but there is currently insufficient evidence of its outcome to permit its recommendation. According to the recent consensus guideline, single-stage revision also does not have sufficient evidence to warrant recommendation. A decision to perform a single-stage revision should be reached following multidisciplinary team discussion and should only be considered in the rare circumstance where a two-stage revision is thought to cause excessive morbidity to the host and the infection is known to be caused by a low virulence pathogen with a favourable antibiogram [229].

Although there has much been progress in the standardisation of current management strategies in PJI, there is potential for far greater improvement, particularly in the context of non-hip and knee PJI. It is hoped that national infection registries [231] and well-designed controlled trials [175] will address the collective shortcomings in the current understanding of available treatment strategies. Furthermore, with demonstrably better functional and patient-reported outcomes following DAIR and single revision, it should be both a research and clinical priority to seek modalities that will optimise the effectiveness of lower morbidity treatment options.

8.9 Novel Treatment Strategies

8.9.1 Organisational Innovations

Hospital and surgeon volume have been shown to improve outcomes on primary arthroplasty, including decreased morbidity, mortality and length of in-patient stay [232–234]. The critical thresholds in primary arthroplasty are > 35 THA and > 20 unicompartmental knee replacements per surgeon per year to see a fall in the risk of postoperative complications [232, 235]. Although there is no evidence in the literature to demonstrate similar effects in the context of PJI, a recent international consensus meeting recommended with 97% agreement amongst delegates that surgeons should be performing > 25 cases of PJI per year [57]; estimates from the literature place the current number of PJI cases performed per surgeon to be between 3 and 50 per annum [125, 236].

The effect of multidisciplinary or interdisciplinary teams (MDT) in prevention of PJI has been widely reported; however there are limited data on its impact on the outcomes of PJI management [57]. Nevertheless, there has been a growing enthusiasm for the implementation of MDT-led management in PJI. Centres such as the Oxford Bone Infection Unit in the UK and Oregon Health and Science University in the USA have successfully developed and implemented MDT models for the care of musculoskeletal infections, including PJIs. Reported outcomes from these centres suggest that MDT and outpatient parenteral antimicrobial therapy services can improve PJI management, not only in the diagnosis and management of PJI but also in reducing recurrence and requirement for in-patient care [99, 237, 238]. Described MDT models include a number of surgical and medical specialities, such as orthopaedic surgery, plastic surgery, anaesthetists, radiologists, infectious disease physicians, clinical microbiologists and internal medicine physicians. Furthermore, ancillary services such as nutrition, physical therapy, pharmacy (including outpatient parenteral antimicrobial therapy), nursing and social care co-ordination (including access to psychological support, e.g. counselling and peer support groups) have been shown to improve overall outcomes [239].

8.9.2 Novel Treatment Modalities

Surgical techniques and antimicrobial delivery systems have been developed, and indications evolved and narrowed in a bid to improve outcomes [63, 240]. With a demonstrable superiority in functional and patient-reported outcomes following single surgery procedures [55, 60, 104, 125], there is a clear clinical need to optimise the lower morbidity treatment options. Greater understanding of the roles bacterial biofilms and intracellular pathogens play in the pathogenesis of PJI has identified novel therapeutic targets with the potential to be readily translated to clinical treatments [80].

Mechanisms of antimicrobial tolerance in biofilms

| Impaired penetration | Altered microenvironment | Stress response | Persister formation |
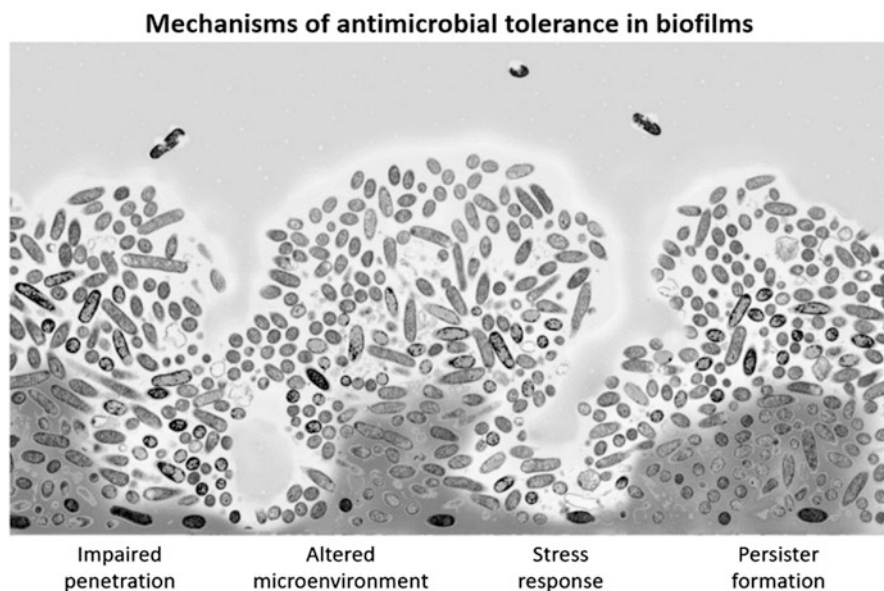
Fig. 8.4 Summary of antimicrobial tolerance mechanisms within bacterial biofilms

Recalcitrant PJI is due to the incomplete eradication of pathogens residing in biofilms or within host cells, which act as a reservoir for prosthesis recolonisation and re-emergence of the infection. Bacteria within biofilms display a tolerance to antimicrobials (Fig. 8.4), which allow them to survive in the presence of antimicrobial and antiseptic agent concentrations that are up to a 1000-fold greater than the typical breakpoints (i.e. minimum inhibitory concentration) used in standard laboratory susceptibility testing [241, 242]. One mechanism for this tolerance is the incomplete penetration of some antimicrobials through the biofilm due to the presence of the glycocalyx [243]. Secondly, the nutrient-deplete environment induces a stress response in bacteria leading to a substantial reduction in cellular growth and metabolic activity in comparison to planktonic phenotypes [244]. Reduced cellular growth and metabolism lead to the development of tolerance as antimicrobials generally exploit targets involved in bacterial reproduction and cell maintenance. This 'biofilm phenotype' has been compared to the sessile state seen in persister cells [245, 246]. The phenomenon of persistence was recognised in the mid-1940s in experiments in which cultures of penicillin-sensitive bacteria survived treatment with penicillin. The subpopulation of surviving bacteria has been referred to as persisters. These transient phenotypic variants go on to exhibit drug susceptibility upon subculture [247].

The ability of some pathogens, such as *S. aureus*, to colonize specialised niches such as biomaterials and connective tissue is attributed to their extensive repertoire of virulence factors that allow them to evade, inactivate and manipulate the host immune system. They have been shown to inhibit elements of both innate and

acquired immunity [248–250]. A further mechanism in the pathogenesis of recalcitrant PJI is the ability of some pathogens to invade host cells [77, 78]. Intracellular persistence allows pathogens to avoid antimicrobial exposure, as well as innate and acquired immune processes. Furthermore, some pathogens can go on to induce apoptosis of the host cell, allowing the bacteria to colonize implants and connective tissue [251].

Current investigational therapeutic technologies can be divided into the following: (1) inhibition of transmission, (2) anti-adhesion strategies, (3) metabolic modulation, 4) biofilm dispersion, (5) novel antimicrobial agents, (6) immunotherapy and (7) intracellular penetration (Table 8.2).

8.9.2.1   Inhibition of Transmission

Preoperative skin and nasal decolonisation with topical antiseptic solutions and antibiotics, most commonly mupirocin, has been shown to be effective [252–254]. One limitation of these regimens is low patient compliance. One study in elective cardiothoracic and orthopaedic patients reported compliance to be as low as 39% [255]. Reasons for limited compliance include the intensity of decolonisation protocols, especially when carried out at the patient's home, and the poor tolerance of nasal mupirocin by patients [256]. A further argument against the universal use of decolonisation regimens is the potential for the development of antimicrobial resistance [257]. One study reported that prior topical mupirocin use increased the risk of mupirocin resistance in methicillin-resistant *S. aureus* (*MRSA*) carriers by ninefold [258]. A systematic review has reported that the widespread use of mupirocin

Table 8.2 Potential therapeutic classes in the prevention and management of prosthetic joint infections

Therapeutic class	Potential technology
Inhibition of transmission	Non-antimicrobial decolonisation regimens [261, 262]
	Environmental bactericidal phototherapy [263, 264]
Anti-adhesion	Biomaterial surface modification [245, 265, 266]
	Surface-bound antimicrobials [267, 268]
Metabolic modulation	Metabolic stimulation [313–316]
	Stress response inhibition/manipulation [310–312]
Biofilm dispersal	Enzyme therapy [344–346]
	Passive immunisation [347–349]
	Physical therapies [350–354]
	Quorum sensing manipulation [403–407]
Novel antimicrobial agents	Antimicrobial peptides [408, 409, 413]
	Bacteriophages [435, 436, 476]
Immunotherapy	Active immunisation [245, 452, 453]
Intracellular penetration	Cell penetrating peptide addition [474, 475]
	Liposome encapsulation [298, 299]

for decolonisation is associated with ~ 1% risk for the development [259]. The relevance of mupirocin resistance to development of healthcare-associated infection is uncertain, but it remains the chief concern limiting its widespread use in this context, leading to the exploration of alternative decolonisation strategies [260]. Potential alternative therapies to topical antimicrobial decolonisation include photodynamic therapy [261] and near infrared phototherapy [262]. These therapies utilise the infrared component of the electromagnetic field to inactivate commensal organisms, with the potential for pathogenic transformation (e.g. *S. aureus*), that reside in the nasal epithelial. Photodynamic therapies combine phototherapy with a photosensitiser, such as methylene blue, which is activated by the light to form cytotoxic free radicals, leading to bacterial eradication. A further application of phototherapies is to use it in a continuous ambient mode to decontaminant surgical fields and healthcare environments [263]. High-intensity narrow spectrum light (405 nm) when incorporated into the lighting units has been shown to be effective in the decontamination of isolation rooms within critical care wards [264].

8.9.2.2 Anti-adhesion

Strategies aimed at preventing bacteria from adhering to biomaterial surfaces to prevent biofilm formation have shown potential in preclinical development. This can be accomplished by changing the physical properties of prosthesis and implant surfaces [245, 265], facilitating the attachment of host cells to inhibit bacterial adhesion competitively [266] or integrating antimicrobial agents (e.g. nanoparticles and antimicrobial peptides) [267, 268]. Nonspecific inhibition of adhesion is generally obtained by manipulating the hydrophobicity [269], topography [270–272] and chemical charge [273, 274] of prosthetic surfaces. The effect of surface hydrophobicity on bacterial adhesion depends on the hydrophobicity of the bacterial cell. Bacteria with a more hydrophobic cell surface preferentially colonize hydrophobic materials and vice versa [275]. In recent years attempts have been made to develop superhydrophobic surfaces [269]. Inspired by lotus leaves, dragonfly wings and shark skin [269], novel surfaces with nanopatterned structures and very low affinity for water have been developed to prevent colonisation against a variety of bacterial species [276, 277]. Surface topography at the micrometre and nanometre scale is an important determinant of bacterial attachment [278]. Micropatterning has been shown to favour bacterial adhesion by not only increasing the contact area between the bacteria and the implant surface, but it also reduces the shear stress experienced by attached cells, as well as the implant's hydrophobicity [270–272]. In contrast, nanopatterning of surfaces has been shown to impair bacterial adhesion when the patterning on the surface is smaller than the size of the bacterium [272, 277, 279]. However, the effectiveness of current designs is thought to be species-specific, as both cell shape (spherical staphylococci versus rod-shaped *Pseudomonas, Aeruginosa* and *Escherichia coli*) [280] and the composition of the cell envelope (Gram-positive staphylococci versus Gram-negative *P. aeruginosa* and *E. coli*) [281] are thought to be important factors in bacterial cell interaction with

nanopatterns. Investigations using self-assembled polymer monolayers have shown that specific chemical groups such as hexa(ethylene glycol), tri(sarcosine), N-acetylpiperazine and intramolecular zwitterions on solid substrates affect attachment behaviour of PJI pathogens such as *S. aureus* and *S. epidermidis* [265, 274, 282].

An efficient anti-adhesive strategy should not only limit bacterial protein adherence but also host-protein interaction in order to avoid the formation of a conditioning film, which facilitates bacterial colonisation. Molecules, such as non-leaching polymeric sulphobetaine, which act as a wetting agent, have been shown to reduce host protein and cellular adhesion, as well as microbial attachment in vitro and in vivo [283]. The glycocalyx-like molecule methyl-cellulose has also been shown to display anti-adhesive properties for both eukaryotic cells and bacteria by utilising biomimetic properties. It has been used to coat totally implanted venous access ports. Coated devices implanted in rats have been found to resist *P. aeruginosa* and *S. aureus* adhesion, reducing biofilm formation, as well the attachment of infective thrombi [284].

A further surface-modifying approach is the addition of antibacterial nanoparticles or peptides through direct bonding to prostheses or utilising carriers [267]. Nanoparticles are versatile and are becoming increasingly popular as a biofilm-targeting approach. Nanoparticles with intrinsic antimicrobial activity, primarily inorganic materials such as silver, can act as anti-biofilm-targeting agents or as nano-coatings. The antimicrobial action activity of these agents is related to perturbations in bacterial cell membranes, disruption of ATP-associated metabolism and the generation of cytotoxic hydroxyl radicals [285, 286]. Silver-based implant coatings (non-nanoparticle) have shown potential in endoprostheses after segmental bone resection and fracture-related infections [89, 268, 287]. In a retrospective case-control series, silver-coated endoprostheses have been found to be associated with a ~ 50% relative risk reduction in postoperative infection following primary tumour surgery, as well as improved infection eradication following debridement and implant retention, compared with uncoated titanium tumour prostheses [287]. Silver has also been evaluated as an additive component in urinary catheters, vascular grafts and endotracheal tubes with varying degrees of success [288, 289]. Additive manufacturing and nanoparticle incorporation technologies have been developed to minimise off-target effects and systemic toxicity [290]. Nanoparticles with multimodal or inducible activation following specific stimuli similar to smart surfaces represent the most widely developed class of nanoparticles currently under development. Recent studies with inorganic nanoparticles, such as iron oxide (Fe_3O_4), with a peroxidase-like function-catalysed hydrogen peroxide (H_2O_2) at concentrations ranging from 0.1 to 1% H_2O_2 in a dose-dependent and pH-dependent manner, have shown potent effects against in vivo biofilms of oral pathogens [291]. Under acidic conditions, nanoparticles activated the generation of free radicals from H_2O_2 in situ, which induced the degradation of the biofilm matrix and the rapid killing of the embedded bacteria (> 5-log reduction of viable cells compared with control cells within 5 min and 5000-fold more effective than 1% H_2O_2 alone) [291]. Covalent bonding of antimicrobials and antifungals to titanium has been reported to

reduce *S. aureus* and *Candida albicans* biofilm formation on implants in vitro without impairing osseointegration and host cell attachment in vivo [266]. Antibiotic carriers such as hyaluronic-based hydrogels or phosphatidylcholine-based materials have been explored as temporary antibiotic-eluting coatings to prevent biofilm formation on implants [292–294]. Observations that hyaluronic-based compounds displayed antibacterial adhesion and antibiofilm forming properties prior to their hydrolytic degradation in vivo initially led to the development of the hydrogels as stand-alone products [295]. More recently, they have been adapted to elute antibacterial agents during their degradation within 72 h of implantation. The elution concentrations of hydrogel-incorporated antimicrobials have been shown to be hundreds or thousands of times higher than their minimum inhibitory concentration [294]. Use of antimicrobial-loaded hydrogels have been found to carry a tenfold relative risk reduction in early surgical site infections following primary and revision joint arthroplasty in a European multicentre randomised controlled trial [296]. One hydrogel product currently has European regulatory approval for clinical use in joint replacement and fracture fixation surgery [294]. Owing to their flexible chemical structures, nanoparticles can also function as drug delivery vehicles (nanocarriers), with organic nanoparticles accounting for over two-thirds of the systems approved for use in humans [297]. Liposomes are vesicles that are composed of one or more phospholipid bilayers. They are one of the most widely developed organic nanoparticles for drug delivery. They are able to penetrate biofilms, are biocompatible and show efficacy against biofilms of a wide range of bacterial species for a diverse number of antibiotics [298, 299]. These nanocarriers can protect the antimicrobial agent from deleterious interactions with the matrix or enzymatic inactivation and degradation at the infection site by bacterial virulence factors or host components. The lipid structure can also fuse with the bacterial outer membrane, releasing the drug directly into the cell, thereby potentially maximising therapeutic effects while reducing host cytotoxicity [299]. Furthermore, liposomes can carry more than one drug by co-encapsulation and can also be functionalised by linking biomolecules (e.g. peptides and pH-responsive polymers) on the nanoparticle surface to increase targeting specificity and triggered release. Several formulations are currently in preclinical studies and clinical trials, and some are commercially available [300]. Water-soluble polymeric nanocarriers have been used to encapsulate hydrophobic and apolar drugs into aqueous solution. Similarly, nanoparticles conjugated with a pH-responsive element [301] or pH-sensitive surface charge switching [302] have been developed to increase biofilm penetration and selective bacterial binding for targeted delivery and antibacterial activity in acidic conditions [303].

Multifunctional surfaces that incorporate host tissue integration, bactericidal properties and anti-adhesive activity are currently undergoing clinical translation [304–306]. One example of such a strategy, showing promising in vitro activity, are anti-adhesive polymer brushes. These are composed of the co-polymer pluronic F-127 functionalised with antimicrobial peptides and arginine-glycine-aspartate peptides, which confers antibacterial adhesion and bactericidal activity and promotes the adhesion and spread of host tissue cells, respectively [307].

8.9.2.3   Metabolic Modulation

There has been a focus on strategies to reverse the cell dormancy associated with the persister and biofilm phenotypes. With growing evidence that one of the main factors leading to persister formation and dormancy is nutritional stress [308, 309], preclinical investigations have focused on the inhibition and manipulation of the cellular stress responses [310–312], as well as metabolic stimulation [313–316]. One major problem caused by biofilms is their increased tolerance towards current antimicrobial agents, limiting their effectiveness in the treatment of PJI in clinical settings [317]. The clinical emergence of antimicrobial resistance to common PJI pathogens, such as *S. aureus*, has led to the modifications of systemic antibiotic regimens. Rifampicin inhibits DNA replication and exhibits bactericidal activity against biofilm-forming microorganisms; however, rapid resistance develops when used as a monotherapy. In vitro and in vivo studies suggest that addition of rifampicin to current standard of care systemic antibiotics reduces colony-forming units in infected periprosthetic tissues and may reduce biofilm formation [54, 318–320]. However, some studies have found rifampicin to be antagonistic with some antimicrobials such as gentamicin [65], linezolid and clindamycin [321]. The presence of a sub-inhibitory concentration of DNA synthesis-inhibiting antibiotic (e.g. rifampicin) with the addition of a protein synthesis inhibitor (e.g. gentamicin, linezolid and clindamycin) has been found to increase the steady-state growth rate of planktonic *E. coli* and *S. aureus* cultures [322, 323]. It has been shown that there is an optimal ratio between inhibition of protein synthesis and DNA replication. It is only when one of these processes is sufficiently hampered that there is growth inhibition of planktonic bacteria. Therefore, using protein synthesis inhibitors and DNA synthesis inhibitors in combination could produce higher growth than just a single inhibitor by itself, leading to an antagonistic interaction [324]. Furthermore, it has been suggested that bacteriostatic antibiotics lead to a reduction in metabolic activity and cellular stress responses [312, 325, 326], which are already inhibited in the biofilm state, reducing antimicrobial uptake and induced oxidative damage [65, 327, 328], resulting in tolerance of bactericidal antimicrobials [329, 330].

Persister cells have been proposed as an additional innate mechanism for biofilm antibiotic resistance [331]. The physiology *of* biofilm-associated bacterial cells bears striking similarity to that of persister cells. Cells that detach from antibiotic-tolerant biofilms and grow planktonically also revert to a drug-susceptible state [332, 333]. Stationary phase (i.e. nutrient limited) cultures of *S. aureus* also demonstrate remarkable antibiotic tolerance [334–337]. By definition, stationary phase cells are slow- or non-growing, a characteristic shared by biofilm cells and persister cells. Cells in such a metabolically inactive state are inherently more tolerant to antimicrobial drugs that target actively growing cells. For example, beta-lactam antibiotics are ineffective against cells that are not actively dividing and synthesising new cell wall peptidoglycan [338]. Like biofilm-associated bacteria, cells from stationary phase cultures also exist in a high cell density environment. At high cell densities, cells are likely to become starved of nutrients, oxygen or both, resulting in a drop in intracellular ATP. It has been reported that intracellular ATP

concentration appears to be the major determinant of survival to an antibiotic challenge for both stationary phase cells and persister cells in *S. aureus* [337]. The same may also be true for biofilm-associated cells. The limited nutrient and oxygen availability within the biofilm presumably results in reduced metabolic activity and a lower energy state, which is a hallmark of persister cells that can survive exposure to most bactericidal antibiotics. It may be that low cell energy levels are the major determinant of antibiotic tolerance in biofilm cells, persister cells and stationary phase cells. For example, *S. aureus* initiates expression of biofilm adhesins in response to a variety of external stresses, including nutrient limitation, pH stress, osmotic stress and sublethal antibiotic challenge [339–341]. Thus, biofilm formation may also be viewed as a response by the bacteria to environmental stress that not only promotes intercellular adherence but also imposes a selective pressure for metabolically inactive, energy-depleted cells that can survive hostile growth conditions, including antibiotic challenges [342]. Metabolic stimulating strategies have been explored to potentiate antimicrobial activity. Glucose supplementation has been used to potentiate gentamicin- and daptomycin-induced killing of *S. aureus* persisters by increasing antimicrobial penetration through upregulation of active bacterial membrane transportation [313, 314, 316]. Alkalinisation by basic amino acids such as L-arginine has also been reported to enhance aminoglycoside action against in vitro and in vivo biofilms and persisters [315]. Stress-induced persister formation is mediated by transcription factors such as ppGpp [308, 309]. Strategies targeting these stringent response factors have been found to display anti-biofilm activity. The *S. aureus* stringent response inhibitor, the peptide 1018 (VRLIVAV-RIWRR-NH$_2$), has been shown to demonstrate in vitro activity against *P. aeruginosa* and *S. aureus* biofilms by inducing ppGpp degradation [310]. In addition, they have been shown to have a potentiating effect on ciprofloxacin when used to treat in vitro biofilms [311]. A further strategy is to exploit the dormancy displayed by persister and biofilm-associated cells. Low cellular activity predisposes Gram-positive pathogens to proteolysis induced by a novel acyldepsipeptide antimicrobial, ADEP4. It activates the nonspecific ClpP protease in Gram-positive pathogens in an ATP-independent manner [334]. ADEP4 has been shown to be effective as a sole agent and displayed potentiation of rifampicin against in vivo persisters, stationary phase cells and biofilms in a murine infection model [334].

8.9.2.4 Biofilm Dispersion

A further area of research has been on the induction of biofilm dispersal, as antimicrobial tolerance has been shown to be reversed following dispersion [343]. Early efforts have focused on the utilisation of enzyme therapies [344–346]; passive immunotherapy, which utilizes monoclonal antibodies against components of the glycocalyx [347–349]; and physical modalities, such as ultrasound [350] and pulsed electromagnetic fields [351–354] to disperse the bacteria from the biofilm. A further method of dispersion which has been investigated more recently is based on the manipulation of quorum sensing mechanisms [355].

Enzymatic treatments such as proteinase K, trypsin, dispersin B, lysostaphin, DNases and fibrinolytics have shown promise in their ability to disperse staphylococci from biofilm [344–346]. They have been used in combination with antimicrobial agents to target the detached cells [356, 357]. Biofilm-degrading enzymes, such as dispersin B, DNase I, fibronolytics and lysostaphin, have been shown to reduce the glycocalyx mass and biofilm-associated cell numbers [357–361]. Dispersion B is an enzyme discovered in *Aggregatibacter actinomycetemcomitans* and acts on methicillin-sensitive *S. aureus (MSSA)* biofilms by hydrolysing the polysaccharide intercellular adhesin, which is a key factor in biofilm formation [346]. Donelli et al. [362] reported that dispersin B alone or in combination with a second-generation cephalosporin (cefamandole nafate) hydrolysed the glycocalyx of a staphylococcal biofilm, promoted antibiotic penetration and potentiated the bactericidal effect of antimicrobials. Furthermore, dispersin B has been found to act synergistically with triclosan when used against *S. aureus* biofilms formed on vascular catheters [356]. The purified recombinant DNase I derivative (DNase1L2), extracted from human stratum corneum, has been reported to eradicate biofilm-associated *P. aeruginosa* and *S. aureus* effectively [358]. Treatment of *S. aureus* biofilms with combinations of recombinant human DNase I (rhDNase I) and topical antiseptics (chlorhexidine gluconate and povidone iodine) demonstrated effective eradication compared to treatment with antibiotics only [363]. It has been hypothesised that DNase not only induces dispersion of biofilm-associated cells but also alters the topography and morphology of the glycocalyx [364]. However, more established *P. aeruginosa* biofilms have been shown to be refractory to DNase I. It is thought that the production of high quantities of glycocalyx and proteolytic exo-enzymes by the mature *P. aeruginosa* biofilms inactivated DNase I [360]. Fibrinolytics such as streptokinase or nattokinase break down the fibrin matrix within biofilm and decrease the effective biofilm eradication concentration of available systemic antibiotics [346, 365]. Lysostaphin is a naturally secreted bacteriocin, comprising a peptidoglycan-dependent endopeptidase encoded on a native plasmid of *Staphylococcus capitis* [366], a natural environmental competitor of *S. aureus*. It selectively and efficiently degrades pentaglycine cross-links in the peptidoglycan cell wall of *S. aureus* and coagulase-negative *S. epidermidis,* ultimately resulting in bacterial lysis and death. Lysostaphin is known to be highly specific to staphylococcal species. It rarely targets unrelated bacteria, reducing the risk of promoting unwanted resistance in non-pathogenic commensal strains, as frequently occurs with broad-spectrum antibiotics. Lysostaphin (15 mg/kg) combined with nafcillin (50 mg/kg) has been reported to kill *MRSA* in biofilms that have developed on vascular catheters, effectively [367]. Lysostaphin has shown synergy when used in combination with commonly administered antibiotics against *MRSA* [368]. Despite the high cost of production, biofilm-eradicating enzymes could possibly be used as an alternative or as a synergistic helper to antibiotics in the treatment of persistent infections [369]. One engineered (bacterio)phage enzyme (peptidoglycan) endolysin, Staphefekt™, developed by the Dutch biotech company Micreos, has been licenced for topical use in humans, for the early stages of *S. aureus*-related skin infections, such as eczema, acne and

rosacea, resulting in a reduction of inflammatory symptoms. The product was approved in the EU under the status of 'medical device' [370].

Passive immunisation strategies targeting components of biofilm, such as extracellular DNA, virulence factors and adhesion factors, have been found to disperse established *S. aureus* biofilms effectively [347]. Monoclonal antibodies raised to target DNA binding proteins which are conserved across many bacterial species, including *S. aureus* [348]. These monoclonal antibodies in combination with daptomycin systemic therapy have been found to display a synergistic effect in both planktonic and biofilm-associated bacteria in a murine implant-associated infection model [348]. Monoclonal antibodies to α-toxin and clumping factor A (ClfA) have been shown to not only inhibit biofilm formation but induce dispersion [349]. The combination of the two monoclonal antibodies resulted in decreased *MRSA* colony-forming units from bone/joint tissue, reduced propensity for infection and less biofilm aggregates in a murine model of haematogenous *MRSA* infection [349].

Physical and mechanical therapies as debridement adjuncts in the treatment of infected implants are another strategy to eradicate bacterial biofilms. Local treatments available in the operating room include topical antiseptic agents and heat. Monotherapy of commonly used topical adjuvant treatments for prosthetic joint infections such as Betadine, Dakin's solution (sodium hypochlorite) or hydrogen peroxide (H_2O_2) has been shown to be only partially effective in the eradication of bacterial biofilms [371]. Acetic acid, commonly found in vinegar, has been used in the treatment of infection since the time of Hippocrates [372]. It is a weak organic acid that is active against Gram-positive and Gram-negative organisms [373–376]. Previous studies have demonstrated its inhibitory and eradication action against bacteria in both planktonic and biofilm states [375, 376]. Clinically, it has been described in the treatment of ear infections [377], burn wounds [378] and catheter-associated urinary tract infections [373]. It has US Food and Drug Administration approval for the therapeutic use of a 0.25% solution in bladder irrigation and a 2% solution for treating otitis externa [375]. A recent study demonstrated that it had an acceptable safety profile and patient tolerance when used as an debridement adjunct in periprosthetic joint infections [74]. Leary et al. showed that the combination of 4% chlorhexidine with autoclave and scrubbing was able to remove over 99% of established *S. aureus* and *S. epidermidis* biofilms on cobalt chromium discs [379]. An evolution of this strategy is the development of non-contact induction heating of metal implants. Induced heating metal implants causes thermal damage to the biofilm, resulting in bacterial eradication. Furthermore, the heat from induction acts synergistically with antibiotics [380]. It uses pulsed electromagnetic fields to induce eddy currents within metallic prostheses. These eddy currents are electrical currents within the metallic object that oppose the change in the induced electromagnetic field, resulting in generation of heat energy, as derived from Faraday's law of electromagnetic induction [381]. In vitro studies have shown it to be effective in reducing the bacterial load within clinically feasible parameters [382–385]. Direct current therapy has also been explored using a cathodic voltage-controlled electrical stimulation to titanium with an established bacterial biofilm. Early in vivo animal studies have reported direct current to be effective at reducing both planktonic and

biofilm-associated *MRSA* [386]. Pulsed electromagnetic fields can also be modulated to have a nonthermal effect similar to direct current, with the advantage of being applied transcutaneously. They have been reported to enhance the efficacy of biocides and antibiotics in killing biofilm bacteria to levels very close to those needed to kill planktonic bacteria [354, 387]. In vitro experimentation has replicated this effect in *S. epidermidis* [354], and *P. aeruginosa* biofilms are promising, especially when in combination with existing antibiotics [351–353]. Bacterial inactivation using ultrasound treatment was first reported in the 1920s [388], and the investigation on the mechanism of microbial inactivation began in the 1960s [389]. The mechanism of microbial killing was thought to be mainly due to thinning of cell membranes, localised heating and production of free radicals [390]. Ultrasound technologies applied to antibacterial treatment have already been extensively developed to play a key role for their future use in the food industry as well as for water decontamination [391, 392]. However, the acoustic parameters used to achieve these effects are known to cause collateral damage to host tissues [393]. Low-intensity pulsed ultrasound therapy currently has regulatory approval for its use in the management acute fractures [394]. Its use in the potentiation of antimicrobials when used against biofilms has also been investigated. Carmen et al. found that low-intensity ultrasound potentiated vancomycin activity against *S. epidermidis* biofilm infections in a lapine model [395]. Moreover, low-frequency ultrasound therapy has been shown to increase the in vitro elution of antibiotics from antibiotic-loaded polymethylmethacrylate bone cement, the most commonly used local antimicrobial depot in PJI, without compromising its mechanical integrity [396]. Although the potential for ultrasound as an antimicrobial therapy has been reported in vitro and in vivo animal models [397, 398], it has yet to be adopted into clinical practice. More recent studies have shown that even at frequencies and intensities insufficient to achieve bacterial eradication, they can still produce tissue damage [399, 400].

Further studies, focusing on identifying the cellular processes affected by ultrasonic fields, as well as quorum sensing and gene expression, would allow better understanding of the action of sublethal ultrasound frequencies. Despite the growing body of literature describing the effects of ultrasound activity against planktonic bacteria and biofilms, its full effect still requires further clarification. The studies carried thus far have provided sufficient analysis of ultrasound diffusion and macroscopic response by bacteria in planktonic and biofilm form for some species. Further analysis of cell metabolism and membrane transport activity in response to ultrasonic fields would represent the next step in the path from the laboratory bench to the surgical bedside for therapeutic ultrasound.

Finally, quorum sensing systems have also been identified, in recent years, as a potential therapeutic target to trigger biofilm dispersal. Quorum sensing regulates a host of bacterial virulence behaviours, including biofilm formation [401]. Quorum sensing compounds include N-acyl-homoserine lactones, produced by Gram-negative bacteria, and autoinducing peptides (e.g. autoinducer 2) produced by Gram-positive bacteria. Inhibition of biofilm formation by quorum sensing quenchers or inhibitors has shown potential in preventing biofilm formation by many pathogens. Enzymatic degradation of quorum sensing signals, such as lactonase,

acylase, oxidoreductase and paraoxonase, has shown promising in vitro results in controlling biofilm formation [402]. Quorum sensing quenchers can also attenuate quorum sensing by blocking or shutting down the expression of quorum sensing genes in pathogens, which leads to inhibition of biofilm formation without killing planktonic cells or influencing normal growth. Autoinducing peptide treatment in vitro has been shown to trigger dispersal of *MRSA* biofilms on titanium discs, increasing their susceptibility to antibiotic therapy [355]. Recently, inhibition of quorum sensing and biofilm formation has been reported in several studies [403–405]. One of the most studied quorum sensing quenchers is the RNA III-inhibiting peptide, which has been shown to have with activity against *S. aureus* biofilms [406, 407]. The injection of RNA III-inhibiting peptide in rats with *MRSA* graft infection was found to suppress staphylococcal RNA III-activating protein and *agr* quorum sensing systems, leading to staphylococcal biofilm dispersion and subsequent eradication [404].

8.9.2.5 Novel Antimicrobial Agents

The ability of several novel antimicrobial agents to eradicate biofilm formation effectively on abiotic surfaces has been reported; these include antimicrobial peptides (AMPs) and bacteriophages. AMPs are naturally produced by both eukaryotic and prokaryotic cells as a part of their innate immune/defence systems [408]. The unique features of many AMPs are their small size (15–30 amino acids), charge (positive/cationic) and ability to target cell membranes [408, 409]. The specificity of AMPs can also be manipulated by designing specifically targeted AMPs, highly selective against pathogens but harmless to non-pathogenic bacteria [410, 411]. Many AMPs target the cell wall membrane by either inducing pore formation or membrane perturbation [412]. They are bactericidal to both active and sessile bacteria in biofilms [413]. However, at low concentrations, AMPs may also act bacteriostatically [414]. The binding of AMPs to extracellular DNA has been reported to enhance the detachment of biofilms [415]. Furthermore native AMPs have been used as design templates for a large variety of synthetic AMPs, some of which have been evaluated in phase II and III clinical trials [416]. Their antimicrobial effect has been shown to be enhanced by manipulation of their amino acid composition [417–419]. One example led to the synthesis of the broad-spectrum bactericidal peptide R-FV-I16 [419]. There are many more examples of antibiofilm antimicrobial peptides that have recently been consolidated in the specialised biofilm-active antimicrobial peptide database [420]. However, as with most receptor-specific antimicrobial agents, bacteria are able to develop survival adaptions to AMPs. This tolerance is developed through a number of mechanisms, such as mutations that change the structure and charge of the cytoplasmic membrane, modification of lipopolysaccharides in the cell wall and secretion of AMPs by specific efflux pumps [421]; however, this stress response to AMPs can be used to potentiate commonly used antimicrobials in PJI. In *S. aureus* biofilm formation, the regulatory system, GraRS, plays an important role in the microorganism's resistance to AMPs [422].

Staphylococci have a diverse network of regulators that modify gene expression and enable them to tolerate a wide range of environmental stresses which include AMPs and antibiotics. They are able to alter the proportion of the negatively charged polysaccharide intercellular adhesin and positively charged teichoic acids in their extracellular polymeric matrix and cell membrane via the GraRS system [312]. These modifications can confer significant tolerance to both AMPs and positively charged antimicrobials such as gentamicin, vancomycin and daptomycin [329, 330]. In vitro investigations of antibiotic combinations used to treat staphylococcal biofilms have found that combinations of bactericidal cell wall targeting antimicrobials such as daptomycin, gentamicin and vancomycin display synergism [65, 100]. It has been suggested that over-activation of the staphylococcal envelope stress response to dual AMP and/or cell wall targeting antimicrobials may account for this observed synergistic effect [65, 326]. This has been corroborated by further in vitro studies with a synergistic effect on *MRSA* biofilms when nisin was combined with daptomycin/ciprofloxacin, indolicidin with teicoplanin and cecropin-melittin A amide with ciprofloxacin [423, 424]. The combination of the cationic peptides and cell wall-acting antimicrobials (e.g. linezolid and vancomycin) has been found to eradicate *S. aureus* biofilms effectively on venous catheters [425] and vascular grafts [426] in rat implant infection models. Caution should be exercised when attempting to combine cell membrane or wall targeting AMPs with bacteriostatic antimicrobials. An in vitro study found that there was strong antagonism between gentamicin and linezolid, rifampicin and clindamycin when used in combination against staphylococcal biofilms [65]. It has been suggested that the common final pathway for all bactericidal agents is overwhelming oxidative damage from hydroxyl radical formation. While bacteriostatic drugs do not cause oxidative stresses, their effects deplete the pool of redox-active metabolic intermediates such as NAD(H), leading to impaired bactericidal antimicrobial or AMP activity [328]. The potency of antimicrobial combinations is ultimately determined by the synergy of interacting antimicrobials, where each one of them is acting on different but complementary targets. A further limitation is the delivery of AMP therapy and avoiding deactivation by the host immune system. Immobilisation of antimicrobial peptides on surfaces has been performed with a variety of peptides and fixation techniques. For peptides to be effective after immobilisation, they must retain the structural integrity which is critical to antimicrobial activity. Other decisive factors for success are length, flexibility and kind of spacer connecting the peptide to the surface, the AMP surface density and the orientation of the immobilised peptides [427]. Chemical tethering of AMPs to surfaces has been found to decrease their antimicrobial activity or even inactivation in some cases [428, 429]. One approach to overcome this problem is to attach AMPs to hydrogels, which are approved as a surface coating and antibiotic delivery system in orthopaedic surgery [430]. This combination of therapies has been shown to be effective against in vitro *S. aureus*, *S. epidermidis* and *E. coli* biofilms [430]. Controlled release coatings for orthopaedic and trauma devices, for example, are designed to provide a burst release of an antimicrobial agent during the first days after implantation, preferably followed by a continuous release providing local

protective levels during several weeks after implantation. The incorporation of AMPs in such coatings is still in early preclinical development [431].

Bacteriophages are viruses that infect and inactivate bacteria [432]. They have been used to treat bacterial infections since their discovery at the turn of the twentieth century [432–434]. Translational development of bacteriophages as an antimicrobial therapy continued in a limited fashion, most notably in the Republic of Georgia, as the wider adoption of antibiotics completely displaced phage therapy in the rest of the world [435]. Each bacteriophage particle contains a nucleic acid genome that is enclosed in a protein or lipoprotein capsid. They are obligate parasites and require a bacterial host in order to replicate. They multiply by means of a lytic cycle in which the bacteriophage particle is adsorbed to the host bacterial cell surface, injects its genomic material and hijacks its host's metabolic machinery, resulting in intracellular bacteriophage replication. The final step in the cycle is the liberation of bacteriophage progeny through lysis of its bacterial host [436]. There has been a renewed interest in bacteriophage therapy due to the recognition of antimicrobial resistance globally [436–438]. Unlike traditional antibiotics, bacteriophage activity is not limited in its effectiveness by bacterial cell dormancy [439] nor is its penetration impaired by the biofilm glycocalyx [440]. In fact, the lytic enzymes used in bacteriophage dispersion, such as depolymerases, have been shown to degrade the glycocalyx promoting bacterial cell dispersion from the biofilm [441]. They have also been shown to potentiate commonly used antibiotics [435]. Potential limitations of bacteriophage therapy include the high specificity of each phage strain, even to the level of bacterial strain (narrowing their spectrum of activity), phage resistance and phage inactivation by the patient's immune system. One possible solution to overcome the high specificity could be the use of 'phage cocktails' that combine different species- or strain-specific bacteriophages, giving broad-spectrum activity against the most common known pathogens [436]. As with antibiotics, resistance to bacteriophages can also develop. However, the mutations that confer resistance to bacteriophages come at such a high biological cost to the bacterial cell that phage-susceptible clones are able to persist within the population [442]. Bacteriophages are antigenic and elicit an immune response in humans, resulting in phage activation. However, studies have shown that the antibody response to bacteriophages is very weak except in cases of previous exposure and residual antibody titres. In vitro studies have suggested that bacteriophages are protected in the relatively immune-deficient environment of the bacterial biofilm [443, 444]. Finally, the design of modified phages with enhanced ability to resist clearance by the cellular immune system has been shown to be feasible [445].

The use of AMPs and bacteriophages in the treatment of PJI is promising, both as a monotherapy and as a potentiating agent for current antibiotics [369, 446]. Although regulatory approval is still awaited for these therapies, multinational collaborative efforts are being made to develop the appropriate legislation to drive their translation into clinical practice [447].

8.9.2.6   Immunotherapy

Immunotherapy is another major area of interest which can complement current treatment options against PJI; most attention has been diverted towards targeting *S. aureus* given its ubiquity in nosocomial infections. The perceived advantage of active vaccines is the robustness of the resulting immunity, which includes both cellular and humoral immunity and the potential of lifelong immunity from the generation of protective memory T cells and B cells. However, the greatest limitation of active vaccination is its unpredictability in individual patients, particularly immune-compromised individuals from those with established comorbidities (i.e. ageing, autoimmunity, obesity and diabetes) [448–451]. Despite efforts, the development of vaccine-based strategies for *S. aureus* infection has yet to progress successfully beyond phase I assessment [245, 452, 453]. Some of these vaccine-based strategies have failed as the targeted bacterial cell wall antigens, such as poly-N-acetyl glucosamine and lipoteichoic acid, are not universally expressed by all strains [245]. One *S. aureus* vaccine that has shown early promise targeted capsular polysaccharides conjugated to a recombinant *P. aeruginosa* exotoxin A. However, ultimately this vaccine was not found to reduce *S. aureus* infections in haemodialysis patients [245, 454]. Another vaccine from Merck (V710) showed preclinical promise by targeting iron-regulated surface determinant B[453]. Unfortunately, V710 did not reduce infection rates or mortality in a phase IIb/III trial, which attempted to prevent *S. aureus* infection following cardiothoracic surgery. Furthermore, patients who did develop a surgical site infection were at greater risk of mortality in the vaccine group, suggesting that this vaccine may have suppressed host immunity against sepsis [452], a particularly pertinent concern in the management of PJI [455]. In order to facilitate the future development of vaccine-based strategies it is critical to develop animal infection models that more faithfully replicate human surgical site infections to elucidate host humoral and cell-based immune responses to *S. aureus* [347]. The most significant barrier to development of a successful vaccine is that in contrast to successful immune technologies, which to date have been exclusively against transient flora, *S. aureus* has co-evolved with mammalian hosts to become a human commensal. Thus, all patients have some prior level of acquired immunity against *S. aureus*. However, the protective versus susceptible nature of an individual's immune response against *S. aureus* at the time of treatment is virtually unknown. Therefore, a major research focus in targeting the immune response is understanding the functional role of specific T cells (cellular immunity) and antibodies (humoral immunity) in *S. aureus* infections. To this end there has been a focus on describing anti-*S. aureus* immune responses in both physiological and pathological situations [455–461] with the aim of elucidating the immune proteome of *S. aureus* [462]. A multiplex immunoassay for characterising a patient's immune response has been developed [178] to identify known *S. aureus* antigens; this has since been used to determine if certain antigens dominate humoral immunity in a pilot study of patients with osteomyelitis versus uninfected controls [455].

8.9.2.7 Cellular Internalisation

A further mechanism of bacterial persistence and recalcitrance in PJI is intracellular persistence within host cells [78, 463]. Once considered to be a strict extracellular pathogen, it is now accepted that common PJI pathogens, such as *S. aureus,* can survive within eukaryotic cells, in both professional phagocytes [464–467] (e.g. macrophages and osteoclasts) and non-professional phagocytes (e.g. epithelial cells, endothelial cells and osteoblasts) [465, 468–471]. Inside the cell pathogens can avoid antimicrobial exposure and the host immune system. The intracellular pathogen eventually induces apoptosis of the host cell, allowing it to colonize biomaterial surfaces and connective tissue niches [251]. Attempts to modify antimicrobial agents to target intracellular infections have been reported. Lehar et al. created an antibody-antibiotic conjugate that consists of a monoclonal antibody bound to rifampicin that recognizes the alpha-O-linked N-acetylglucosamine sugars on wall teichoic acids [472]. This antibody-antibiotic conjugate binds to the surface of Gram-positive pathogens; upon opsonisation, the proteolytic environment of the phagolysosome within the host phagocyte activates the attached antibiotic molecule [472]. This antibody-antibiotic conjugate has been reported to have superior bacterial eradication versus systemic vancomycin alone in a murine *MRSA* bacteraemia model [472]. An alternative strategy is the addition of cell penetrating peptides to established antimicrobials and more novel therapies, such as nanoparticles, AMPs and bacteriophages [473]. The addition of these peptides allows the agents to penetrate eukaryotic cells, facilitating mammalian cell internalisation and thereby co-localising the antimicrobial agent with the pathogen [474, 475]. A further approach is the development of liposome nanocarriers. Liposomes, as described earlier in this review, are phospholipid vesicles that are able to penetrate biofilms and mammalian cells. They have been shown to be compatible with a wide range of established antimicrobials commonly used in PJI [298, 299].

8.10 Conclusion

PJIs are not amenable to current antimicrobial treatments or single 'magic bullet' approaches. Recalcitrance is a consequence of complex physical and biological properties with multiple microbial genetic, molecular and physical factors. Importantly, PJIs reflect an interplay between the host and opportunistic pathogens, often within a complex microbiota. Polymicrobial PJIs pose an additional challenge, requiring antimicrobials that are effective against all pathogenic microorganisms in the biofilm and limiting the efficacy of species-specific biofilm-targeting strategies. All of these challenges contribute to the reason why so few therapies have yet to be translated into clinical practice [303]. It has been suggested that the treatment of biofilm infections should take a similar approach to cancer therapy, using combination therapies or those that target more than one component of the complex multicellular microenvironment of PJI [477].

Despite the progress made in the understanding of the pathophysiology of PJI and the identification of potential therapeutic targets, very few non-drug antimicrobial therapies and strategies have progressed beyond preclinical investigation. Conventional clinical treatment has shown little progress beyond the traditional tenets of surgical debridement, irrigation +/− excision, plus local and systemic antimicrobial drug therapy. The urgency to bridge this lag in the translation of basic science understanding to clinical therapies is greater than ever, especially in light of the looming global crisis of antimicrobial resistance [478, 479], which threatens to halt elective joint replacement procedures [437, 480].

References

1. Williams, S. N., Wolford, M. L. & Bercovitz, A. *Hospitalization for Total Knee Replacement Among Inpatients Aged 45 and Over: United States, 2000-2010 Key findings*. (2000).
2. Wolford, M. L., Palso, K. & Bercovitz, A. *Hospitalization for Total Hip Replacement Among Inpatients Aged 45 and Over: United States, 2000-2010 Key findings*. (2000).
3. Canadian Joint Replacement Registry Annual Report. (2018).
4. *Australian National joint replacement registry annual report*. (2018).
5. Hooper, G., J-J Lee, A., Rothwell, A. & Frampton, C. *Current trends and projections in the utilisation rates of hip and knee replacement in New Zealand from 2001 to 2026. Journal of the New Zealand Medical Association NZMJ* **29**, (2014).
6. National Joint Registry for England, Wales, N. I. and the I. of M. *NJR 15th annual report*. (2018).
7. Scottish Arthroplasty Project. *Scottish Arthoplasty Project Annual report*. (2018).
8. OECD. Hip and knee replacement. in *Health at a Glance 2017: OECD Indicators* (OECD Publishing, 2017). doi:https://doi.org/10.1787/5k49h4p5g9mw-en
9. Kurtz, S., Ong, K., Lau, E., Mowat, F. & Halpern, M. Projections of Primary and Revision Hip and Knee Arthroplasty in the United States from 2005 to 2030. *J. Bone Jt. Surg.* **89**, 780–785 (2007).
10. Ackerman, I. N. *et al.* The projected burden of primary total knee and hip replacement for osteoarthritis in Australia to the year 2030. *BMC Musculoskelet. Disord.* **20**, 90 (2019).
11. Inacio, M. C. S., Graves, S. E., Pratt, N. L., Roughead, E. E. & Nemes, S. Increase in Total Joint Arthroplasty Projected from 2014 to 2046 in Australia: A Conservative Local Model With International Implications. *Clin. Orthop. Relat. Res.* **475**, 2130–2137 (2017).
12. British Orthopaedic Association. Getting It Right First Time. (2015). Available at: https://www.boa.ac.uk/pro-practice/getting-it-right-first-time/. (Accessed: 28th June 2017)
13. Kamath, A. F. *et al.* Quantifying the Burden of Revision Total Joint Arthroplasty for Periprosthetic Infection. *J. Arthroplasty* **30**, 1492–1497 (2015).
14. Bozic, K. J. & Ries, M. D. The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. *J. Bone Joint Surg. Am.* **87**, 1746–51 (2005).
15. Public Health England. Surveillance of surgical site infections in NHS hospitals in England. (2016). Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/577418/Surgical_site_infections_NHS_hospitals_2015_to_2016.pdf. (Accessed: 2nd February 2017)
16. Springer, B. D., Cahue, S., Etkin, C. D., Lewallen, D. G. & McGrory, B. J. Infection burden in total hip and knee arthroplasties: an international registry-based perspective. *Arthroplast. today* **3**, 137–140 (2017).

17. Morrison, T. A., Figgie, M., Miller, A. O. & Goodman, S. M. Periprosthetic joint infection in patients with inflammatory joint disease: a review of risk factors and current approaches to diagnosis and management. *HSS J.* **9**, 183–94 (2013).

18. Jaekel, D. J., Ong, K. L., Lau, E. C., Watson, H. N. & Kurtz, S. M. Epidemiology of Total Hip and Knee Arthroplasty Infection. in *Periprosthetic Joint Infection of the Hip and Knee* 1–14 (Springer New York, 2014). doi:https://doi.org/10.1007/978-1-4614-7928-4_1

19. Tissingh, E. K., Sudlow, A., Jones, A. & Nolan, J. F. Orthopaedic surgical site infection surveillance in NHS England. *Bone Joint J.* **99**-B, 171–174 (2017).

20. Pandey, R., Berendt, A. R. & Athanasou, N. A. Histological and microbiological findings in non-infected and infected revision arthroplasty tissues. The OSIRIS Collaborative Study Group. Oxford Skeletal Infection Research and Intervention Service. *Arch. Orthop. Trauma Surg.* **120**, 570–4 (2000).

21. Athwal, G. S., Holmes, S., Diaz, A. P., Faber, K. J. & O'Gorman, D. B. A rapid detection method for Propionibacterium acnes from surgical biopsies of the shoulder. *J. Shoulder Elb. Surg.* **26**, e162 (2017).

22. Achermann, Y., Vogt, M., Leunig, M., Wüst, J. & Trampuz, A. Improved diagnosis of periprosthetic joint infection by multiplex PCR of sonication fluid from removed implants. *J. Clin. Microbiol.* **48**, 1208–14 (2010).

23. Bossard, D. A. *et al.* Optimal length of cultivation time for isolation of Propionibacterium acnes in suspected bone and joint infections is more than 7 days. *J. Clin. Microbiol.* (2016). doi:https://doi.org/10.1128/JCM.01435-16

24. Ascione, T. *et al.* Proceedings of International Consensus Meeting on Orthopedic Infections: General Assembly, Diagnosis, Pathogen Isolation - Culture Matters: International Consensus Meeting on Prosthetic Joint Infection. *J. Arthroplasty* **34**, S197–S206 (2019).

25. Tande, A. J. & Patel, R. Prosthetic joint infection. *Clin. Microbiol. Rev.* **27**, 302–345 (2014).

26. Rosteius, T. *et al.* Evaluating the microbial pattern of periprosthetic joint infections of the hip and knee. *J. Med. Microbiol.* **67**, 1608–1613 (2018).

27. Parvizi, J. *et al.* Resistant organisms in infected total knee arthroplasty: occurrence, prevention, and treatment regimens. *Instr. Course Lect.* **58**, 271–8 (2009).

28. Middleton, R., Khan, T. & Alvand, A. Update on the diagnosis and management of prosthetic joint infection in hip and knee arthroplasty. *Bone Jt. 360* **8**, 5–13 (2019).

29. Malekzadeh, D., Osmon, D. R., Lahr, B. D., Hanssen, A. D. & Berbari, E. F. Prior use of antimicrobial therapy is a risk factor for culture-negative prosthetic joint infection. *Clin. Orthop. Relat. Res.* **468**, 2039–45 (2010).

30. Parvizi, J., Faruk Erkocak, O. & Della Valle, C. J. Culture-Negative Periprosthetic Joint Infection. *J. Bone Jt. Surg. - Am. Vol.* **96**, 430–436 (2014).

31. Pasquaroli, S. *et al.* Antibiotic pressure can induce the viable but non-culturable state in Staphylococcus aureus growing in biofilms. *J. Antimicrob. Chemother.* **68**, 1812–1817 (2013).

32. Pasquaroli, S. *et al.* Role of daptomycin in the induction and persistence of the viable but non-culturable state of Staphylococcus aureus biofilms. *Pathog. (Basel, Switzerland)* **3**, 759–68 (2014).

33. Zhao, X., Zhong, J., Wei, C., Lin, C.-W. & Ding, T. Current Perspectives on Viable but Non-culturable State in Foodborne Pathogens. *Front. Microbiol.* **8**, 580 (2017).

34. Li, L., Mendis, N., Trigui, H., Oliver, J. D. & Faucher, S. P. The importance of the viable but non-culturable state in human bacterial pathogens. *Front. Microbiol.* **5**, 258 (2014).

35. Berbari, E. F. *et al.* Culture-negative prosthetic joint infection. *Clin. Infect. Dis.* **45**, 1113–9 (2007).

36. Oliver, J. D. The Public Health Significance of Viable but Nonculturable Bacteria. in *Nonculturable Microorganisms in the Environment* 277–300 (Springer US, 2000). doi:https://doi.org/10.1007/978-1-4757-0271-2_16

37. Dworkin, J. & Shah, I. M. Exit from dormancy in microbial organisms. *Nat. Rev. Microbiol.* **8**, 890–896 (2010).

38. Kana, B. D. *et al.* The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth *in vitro*. *Mol. Microbiol.* **67**, 672–684 (2008).

39. Whitehouse, J. D., Friedman, N. D., Kirkland, K. B., Richardson, W. J. & Sexton, D. J. The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital: adverse quality of life, excess length of stay, and extra cost. *Infect. Control Hosp. Epidemiol.* **23**, 183–9 (2002).

40. Egol, K. A., Gruson, K., Spitzer, A. B., Walsh, M. & Tejwani, N. C. Do Successful Surgical Results after Operative Treatment of Long-bone Nonunions Correlate with Outcomes? *Clin. Orthop. Relat. Res.* **467**, 2979–2985 (2009).

41. Barker, K. L., Lamb, S. E. & Simpson, A. H. R. W. Functional recovery in patients with nonunion treated with the Ilizarov technique. *J. Bone Joint Surg. Br.* **86**, 81–5 (2004).

42. Poultsides, L. A., Liaropoulos, L. L. & Malizos, K. N. The Socioeconomic Impact of Musculoskeletal Infections. *J. Bone Jt. Surgery-American Vol.* **92**, e13(1)–e13(12) (2010).

43. Fisman, D. N., Reilly, D. T., Karchmer, A. W. & Goldie, S. J. Clinical effectiveness and cost-effectiveness of 2 management strategies for infected total hip arthroplasty in the elderly. *Clin. Infect. Dis.* **32**, 419–30 (2001).

44. Robertsson, O., Ranstam, J., Sundberg, M., W-Dahl, A. & Lidgren, L. The Swedish Knee Arthroplasty Register: a review. *Bone Joint Res.* **3**, 217–22 (2014).

45. Natsuhara, K. M., Shelton, T. J., Meehan, J. P. & Lum, Z. C. Mortality During Total Hip Periprosthetic Joint Infection. *J. Arthroplasty* **0**, (2018).

46. Stanton, T. PJI and Cancer: More Similar Than Different? in *American Association of Orthopaedic Surgeons* (2017).

47. Garrido-Gómez, J. *et al.* Descriptive Analysis of the Economic Costs of Periprosthetic Joint Infection of the Knee for the Public Health System of Andalusia. *J. Arthorplasty* **28**, 1057–1060 (2013).

48. Kapadia, B. H. *et al.* The Economic Impact of Periprosthetic Infections Following Total Knee Arthroplasty at a Specialized Tertiary-Care Center. *J. Arthroplasty* **29**, 929–932 (2014).

49. Kurtz, S. M., Lau, E., Watson, H., Schmier, J. K. & Parvizi, J. Economic Burden of Periprosthetic Joint Infection in the United States. *J. Arthroplasty* **27**, 61-65.e1 (2012).

50. Vanhegan, I. S., Malik, A. K., Jayakumar, P., Ul Islam, S. & Haddad, F. S. A financial analysis of revision hip arthroplasty: The economic burden in relation to the national tariff. *Bone Joint J.* **94**, 619–23 (2012).

51. Kurtz, S. M. *et al.* Infection Burden for Hip and Knee Arthroplasty in the United States. *J. Arthroplasty* (2008). doi:https://doi.org/10.1016/j.arth.2007.10.017

52. Miller, A. O., Henry, M. W. & Brause, B. D. 1 – Prevention of joint infections. in *Management of Periprosthetic Joint Infections (PJIs)* 3–23 (2017). doi:https://doi.org/10.1016/B978-0-08-100205-6.00001-X

53. McPherson, E. J. *et al.* Periprosthetic total hip infection: outcomes using a staging system. *Clin. Orthop. Relat. Res.* 8–15 (2002).

54. Zimmerli, W., Trampuz, A. & Ochsner, P. E. Prosthetic-joint infections. *N. Engl. J. Med.* **351**, 1645–54 (2004).

55. Tsang, S.-T. J., Ting, J., Simpson, A. H. R. W. & Gaston, P. Outcomes following debridement, antibiotics and implant retention in the management of periprosthetic infections of the hip. A review of cohort studies. *Bone Joint J.* **99**, 1458–66 (2017).

56. Haddad, F. S. *et al.* Orthopaedic infection. *Bone Joint J.* **100**-**B**, 1405–1406 (2018).

57. Abblitt, W. P. *et al.* Hip and Knee Section, Outcomes: Proceedings of International Consensus on Orthopedic Infections. *J. Arthroplasty* **34**, S487–S495 (2019).

58. Preobrazhensky, P. M. *et al.* Functional outcome of two-stage reimplantation in patients with periprosthetic joint infection after primary total knee arthroplasty. *Int. Orthop.* 1–7 (2019). doi:https://doi.org/10.1007/s00264-019-04296-z

59. Kuiper, J. W. P. *et al.* Results and Patient Reported Outcome Measures (PROMs) after One-Stage Revision for Periprosthetic Joint Infection of the Hip: A Single-centre Retrospective Study. *J. bone Jt. Infect.* **3**, 143–149 (2018).

60. Grammatopoulos, G. *et al.* Functional outcome of debridement, antibiotics and implant retention in periprosthetic joint infection involving the hip A CASE–CONTROL STUDY. *Bone Jt. J* **99**, 614–22 (2017).

61. Anderson, M. B. *et al.* General Assembly, Treatment, Multidisciplinary Issues: Proceedings of International Consensus on Orthopedic Infections. *J. Arthroplasty* **34**, S239-243 (2019).

62. Mallon, C. M., Gooberman-Hill, R. & Moore, A. J. Infection after knee replacement: a qualitative study of impact of periprosthetic knee infection. *BMC Musculoskelet. Disord.* **19**, 352 (2018).

63. Buchholz, H. W. & Engelbrecht, H. [Depot effects of various antibiotics mixed with Palacos resins]. *Chirurg.* **41**, 511–5 (1970).

64. Buchholz, H. W. *et al.* Management of deep infection of total hip replacement. *J. Bone Joint Surg. Br.* **63**-**B**, 342–53 (1981).

65. Dall, G. F. *et al.* Unexpected synergistic and antagonistic antibiotic activity against Staphylococcus biofilms. *J. Antimicrob. Chemother.* **73**, 1830–1840 (2018).

66. Zimmerli, W., Widmer, A. F., Blatter, M., Frei, R. & Ochsner, P. E. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. *JAMA* **279**, 1537–41 (1998).

67. Frew, N. M., Cannon, T., Nichol, T., Smith, T. J. & Stockley, I. Comparison of the elution properties of commercially available gentamicin and bone cement containing vancomycin with 'home-made' preparations. *Bone Jt. J.* **99**-**B**, 73–77 (2017).

68. Velkov, T., Roberts, K. D. & Li, J. Rediscovering the octapeptins. *Nat. Prod. Rep.* **34**, 295–309 (2017).

69. Khan, W. & Morgan-Jones, R. Debridement: Defining something we all do. *J. Trauma Orthop.* **4**, 48–51 (2016).

70. Kocher, M. S. Early limb salvage: open tibia fractures of Ambroise Paré (1510-1590) and Percivall Pott (1714-1789). *World J. Surg.* **21**, 116–22 (1997).

71. Huntley, J. S. Debridement: Development of the Concept. *J. Perioper. Pract.* **21**, 104–105 (2011).

72. Morgan-Jones, R. Uncemented Revision Total Knee Arthroplasty for Periprosthetic Joint Infection. in *Periprosthetic Joint Infections Changing Paradigms* (eds. Kendoff, D., Morgan-Jones, R. & Haddad, F. S.) (Springer International Publishing, 2016).

73. Lu, M. & Hansen, E. N. Hydrogen Peroxide Wound Irrigation in Orthopaedic Surgery. *J. Bone Jt. Infect.* **2**, 3–9 (2016).

74. Williams, R. L., Ayre, W. N., Khan, W. S., Mehta, A. & Morgan-Jones, R. Acetic Acid as Part of a Debridement Protocol During Revision Total Knee Arthroplasty. *J. Arthroplasty* **32**, 953–957 (2017).

75. Tsang, S. T. ., Gwynne, P. J., Gallagher, M. P. & Simpson, A. H. R. . The biofilm eradication activity of acetic acid in the management of periprosthetic joint infection. *Bone Joint Res.* **7**, 517–523 (2018).

76. Dryden, M., Milward, G. & Saeed, K. Infection prevention in wounds with surgihoney. *Journal of Hospital Infection* **88**, 121–122 (2014).

77. Dusane, D. H. *et al.* Targeting intracellular *Staphylococcus aureus* to lower recurrence of orthopaedic infection. *J. Orthop. Res.* **36**, 1086–1092 (2017).

78. Yang, D. *et al.* Novel Insights into Staphylococcus aureus Deep Bone Infections: the Involvement of Osteocytes. *MBio* **9**, e00415–18 (2018).

79. Atkins, G. J. Osteocytes and periprosthetic joint infection. in *The European Orthopaedic Research Society (EORS) 2018 Meeting* (2018).

80. Tsang, S. T. J. & Simpson, A. H. R. W. Pathogenesis of biomaterial-associated infection. in *Racing for the Surface: Advances in Antimicrobial and Osteoinductive Studies* (eds. Li, B.,

Moriarty, T. F., Webster, T. & Xing, M.) (Springer International Publishing, 2020). doi:https://doi.org/10.1007/978-3-030-34475-7

81. Espehaug, B., Engesaeter, L. B., Vollset, S. E., Langeland, N. & Surgeon, O. Antibiotic prophylaxis in total hip arthroplasty. Review of 10905 primary total hip replacements reported to the Norwegian arthroplasty register, 1987 to 1995. *J Bone Jt. Surg [Br]* **79**, 590–5 (1997).

82. Thierse, L. [Experiences with Refobacin-Palacos with regard to deep late infections following hip-joint endoprosthesis surgery. A 4-years' study (author's transl)]. *Zeitschrift für Orthopädie und ihre Grenzgebiete* **116**, 847–52 (1978).

83. Chiu, F.-Y., Chen, C.-M., Lin, C.-F. J. & Lo, W.-H. Cefuroxime-impregnated cement in primary total knee arthroplasty: a prospective, randomized study of three hundred and forty knees. *J. Bone Joint Surg. Am.* **84**-**A**, 759–62 (2002).

84. Stockley, I., Mockford, B. J., Hoad-Reddick, A. & Norman, P. The use of two-stage exchange arthroplasty with depot antibiotics in the absence of long- term antibiotic therapy in infected total hip replacement. *J Bone Jt. Surg [Br]* **90**, 145–8 (2008).

85. Thomes, B., Murray, P. & Bouchier-Hayes, D. Development of resistant strains of Staphylococcus epidermidis on gentamicin-loaded bone cement in vivo. *J. Bone Jt. Surg. - Br. Vol.* **84**, 758–760 (2002).

86. Anagnostakos, K., Hitzler, P., Pape, D., Kohn, D. & Kelm, J. Persistence of bacterial growth on antibiotic-loaded beads: Is it actually a problem? *Acta Orthop.* **79**, 302–307 (2008).

87. Bálint, L., Koós, Z., Horváth, G. & Szabó, G. Detection of gentamicin emission from bone cement in the early postoperative period following total hip arthroplasty. *Orthopedics* **29**, 432–6 (2006).

88. Fuchs, T., Stange, R., Schmidmaier, G. & Raschke, M. J. The use of gentamicin-coated nails in the tibia: preliminary results of a prospective study. *Arch. Orthop. Trauma Surg.* **131**, 1419–25 (2011).

89. Hardes, J. *et al.* Reduction of periprosthetic infection with silver-coated megaprostheses in patients with bone sarcoma. *J. Surg. Oncol.* **101**, n/a-n/a (2010).

90. Brooks, B. D., Brooks, A. E. & Grainger, D. W. Antimicrobial Medical Devices in Preclinical Development and Clinical Use. in *Biomaterials Associated Infection* 307–354 (Springer New York, 2013). doi:https://doi.org/10.1007/978-1-4614-1031-7_13

91. George, D. A., Gant, V. & Haddad, F. S. The management of periprosthetic infections in the future: a review of new forms of treatment. *Bone Joint J.* **97**-**B**, 1162–1169 (2015).

92. Levack, A. E. *et al.* Current Options and Emerging Biomaterials for Periprosthetic Joint Infection. *Curr. Rheumatol. Rep.* **20**, 33 (2018).

93. El-Husseiny, M., Patel, S., MacFarlane, R. J. & Haddad, F. S. Biodegradable antibiotic delivery systems. *J. Bone Joint Surg. Br.* **93**, 151–7 (2011).

94. Osmon, D. R. *et al.* Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **56**, e1–e25 (2013).

95. Byren, I. *et al.* One hundred and twelve infected arthroplasties treated with 'DAIR' (debridement, antibiotics and implant retention): antibiotic duration and outcome. *J. Antimicrob. Chemother.* **63**, 1264–71 (2009).

96. Tornero, E. *et al.* Importance of selection and duration of antibiotic regimen in prosthetic joint infections treated with debridement and implant retention. *J. Antimicrob. Chemother.* **71**, (2016).

97. Senneville, E. & Coelho, A. AVAPOM: COMPLETE ORAL VERSUS INTRAVENOUS ANTIBIOTIC DOCUMENTED TREATMENT IN PROSTHETIC JOINT INFECTIONS. *Orthop. Proc.* **99**-**B**, (2018).

98. Argenson, J. N. *et al.* Hip and Knee Section, Treatment, Debridement and Retention of Implant: Proceedings of International Consensus on Orthopedic Infections. *J. Arthroplasty* **34**, S399–S419 (2019).

99. Li, H.-K. *et al.* Oral versus Intravenous Antibiotics for Bone and Joint Infection. *N. Engl. J. Med.* **380**, 425–436 (2019).

100. Tsuji, B. T. & Rybak, M. J. Short-Course Gentamicin in Combination with Daptomycin or Vancomycin against Staphylococcus aureus in an In Vitro Pharmacodynamic Model with Simulated Endocardial Vegetations. *Antimicrob. Agents Chemother.* **49**, 2735–2745 (2005).

101. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* (2019).

102. Parvizi, J., Adeli, B., Zmistowski, B., Restrepo, C. & Greenwald, A. S. Management of Periprosthetic Joint Infection: The Current Knowledge. *J. Bone Jt. Surg.* **94**, e104 1 (2012).

103. Lehner, B., Witte, D., Suda, A. J. & Weiss, S. [Revision strategy for periprosthetic infection]. *Der Orthopäde* **38**, 681–8 (2009).

104. Oussedik, S. I. S., Dodd, M. B. & Haddad, F. S. Outcomes of revision total hip replacement for infection after grading according to a standard protocol. *J. Bone Jt. Surg. - Br. Vol.* **92**, 1222–6 (2010).

105. Hansen, E. *et al.* Outcome of one-stage cementless exchange for acute postoperative periprosthetic hip infection. *Clin. Orthop. Relat. Res.* **471**, 3214–22 (2013).

106. Bejon, P. *et al.* Two-stage revision for prosthetic joint infection: predictors of outcome and the role of reimplantation microbiology. *J. Antimicrob. Chemother.* **65**, 569–75 (2010).

107. Yamamoto, P. A., Lahoz, G. L., Takata, E. T., Masiero, D. & Chamlian, T. R. Evaluation of the function and quality of life of patients submitted to girlstone's resection arthroplasty. *Acta Ortopédica Bras.* **15**, 214–217 (2006).

108. Cierny, G., Mader, J. T. & Penninck, J. J. A Clinical Staging System for Adult Osteomyelitis. *Contemp Orthop* 17–37 (1985).

109. Calabrò, F. *et al.* Hip and Knee Section, Treatment, Antimicrobial Suppression: Proceedings of International Consensus on Orthopedic Infections. *J. Arthroplasty* **0**, (2018).

110. Vegari, D. N. & Springer, B. D. Prosthetic Retention: Treatment Options. in *Prosthetic Joint Infections* (eds. Springer, B. D. & Parvizi, J.) 149–158 (Springer New York, 2014).

111. Rand, J. A., Morrey, B. F. & Bryan, R. S. Management of the infected total joint arthroplasty. *Orthop. Clin. North Am.* **15**, 491–504 (1984).

112. Toms, A. D., Davidson, D., Masri, B. A. & Duncan, C. P. The management of peri-prosthetic infection in total joint arthroplasty. *J. Bone Joint Surg. Br.* **88**, 149–55 (2006).

113. Dzaja, I., Howard, J., Somerville, L. & Lanting, B. Functional outcomes of acutely infected knee arthroplasty: a comparison of different surgical treatment options. *Can. J. Surg.* **58**, 402–7 (2015).

114. Konan, S., George, D. A., Punjabi, V. & Haddad, F. S. Acute Infections: Irrigation and Debridement with Implant Retention. in *Periprosthetic Joint Infections: Changing Paradigms* (eds. Kendoff, D., Morgan-Jones, R. & Haddad, F. S.) 135–142 (2016).

115. Buechel, F. F. The infected total knee arthroplasty: Just when you thought it was over. *J. Arthroplasty* **19**, 51–55 (2004).

116. Buechel, F. F., Femino, F. P. & D'Alessio, J. Primary exchange revision arthroplasty for infected total knee replacement: a long-term study. – PubMed – NCBI. *Am. J. Orthop.* **33**, 190–198 (2004).

117. Nguyen, M., Sukeik, M., Zahar, A., Nizam, I. & Haddad, F. S. One-stage Exchange Arthroplasty for Periprosthetic Hip and Knee Joint Infections. *Open Orthop. J.* **10**, 646–653 (2016).

118. Beswick, A. D. *et al.* What is the evidence base to guide surgical treatment of infected hip prostheses? systematic review of longitudinal studies in unselected patients. *BMC Med.* **10**, 18 (2012).

119. Chotanaphuti, T. *et al.* Hip and Knee Section, Treatment, Algorithm: Proceedings of International Consensus on Orthopedic Infections. *J. Arthroplasty* **0**, (2018).

120. Senthi, S., Munro, J. T. & Pitto, R. P. Infection in total hip replacement: meta-analysis. *Int. Orthop.* **35**, 253–60 (2011).

121. Kilgus, D. J., Howe, D. J. & Strang, A. Results of periprosthetic hip and knee infections caused by resistant bacteria. *Clin. Orthop. Relat. Res.* 116–24 (2002).

122. Estes, C. S., Beauchamp, C. P., Clarke, H. D. & Spangehl, M. J. A two-stage retention débridement protocol for acute periprosthetic joint infections. *Clin. Orthop. Relat. Res.* **468**, 2029–38 (2010).

123. Tintle, S. M., Forsberg, J. A., Potter, B. K., Islinger, R. B. & Andersen, R. C. Prosthesis reten-
     tion, serial debridement, and antibiotic bead use for the treatment of infection following total
     joint arthroplasty. *Orthopedics* **32**, 87 (2009).
124. Corona Pérez-Cardona, P. S. *et al.* Clinical experience with daptomycin for the treatment
     of patients with knee and hip periprosthetic joint infections. *J. Antimicrob. Chemother.* **67**,
     1749–54 (2012).
125. Grammatopoulos, G. *et al.* Outcome Following Debridement, Antibiotics, and Implant
     Retention in Hip Periprosthetic Joint Infection—An 18-Year Experience. *J. Arthroplasty* **32**,
     2248–2255 (2017).
126. Haddad, F. S., Muirhead-Allwood, S. K., Manktelow, A. R. & Bacarese-Hamilton, I. Two-
     stage uncemented revision hip arthroplasty for infection. *J. Bone Joint Surg. Br.* **82**,
     689–94 (2000).
127. Haddad, F. S. *et al.* The PROSTALAC functional spacer in two-stage revision for infected
     knee replacements. Prosthesis of antibiotic-loaded acrylic cement. *J. Bone Joint Surg. Br.* **82**,
     807–12 (2000).
128. Windsor, R. E., Insall, J. N., Urs, W. K., Miller, D. V. & Brause, B. D. Two-stage reimplanta-
     tion for the salvage of total knee arthroplasty complicated by infection. Further follow-up and
     refinement of indications. *J. Bone Jt. Surg.* **72**, 272–278 (1990).
129. Colyer, R. A. & Capello, W. N. Surgical treatment of the infected hip implant. Two-stage
     reimplantation with a one-month interval. *Clin. Orthop. Relat. Res.* 75–9 (1994).
130. Kerr, G. J. & Parvizi, J. Knee arthrodesis. in *Prosthetic Joint Infections of the hip and knee*
     (eds. Springer, B. D. & Parvizi, J.) (Springer New York, 2014).
131. Bradbury, T. Resection Arthroplasty and Hip Joint Fusion. in *Prosthetic Joint Infections of the
     hip and knee* (eds. Springer, B. D. & Parvizi, J.) (Springer New York, 2014).
132. Chen, A. F., Fedorka, C. J. & Klatt, B. A. Above-Knee Amputation. in *Prosthetic Joint
     Infections of the hip and knee* (eds. Springer, B. D. & Parvizi, J.) (Springer New York, 2014).
133. Jämsen, E., Huhtala, H., Puolakka, T. & Moilanen, T. Risk factors for infection after
     knee arthroplasty a register-based analysis of 43,149 cases. *J. Bone Jt. Surg. – Ser. A* **91**,
     38–47 (2009).
134. Lenguerrand, E. *et al.* Description of the rates, trends and surgical burden associated with
     revision for prosthetic joint infection following primary and revision knee replacements in
     England and Wales: an analysis of the National Joint Registry for England, Wales, Northern
     Ireland and the Isle of Man. *BMJ Open* **7**, e014056 (2017).
135. Khan, M., Osman, K., Green, G. & Haddad, F. S. The epidemiology of failure in total knee
     arthroplasty. *Bone Joint J.* **98-B**, 105–112 (2016).
136. Moore, A. J., Blom, A. W., Whitehouse, M. R. & Gooberman-Hill, R. Deep prosthetic joint
     infection: a qualitative study of the impact on patients and their experiences of revision sur-
     gery. *BMJ Open* **5**, e009495 (2015).
137. Jacobs, A. M. E., Bnard, M., Meis, J. F., Van Hellemondt, G. & Goosen, J. H. M. The unsus-
     pected prosthetic joint infection: Incidence and consequences of positive intraoperative cul-
     tures in presumed aseptic knee and hip revisions. *Bone Jt. J.* **99B**, 1482–1489 (2017).
138. Chen, A. F., Della Valle, C. J., Rao, N. & Parvizi, J. Treatment of the Infected Total Knee.
     Oper. Tech. Orthop. **22**, 236–246 (2012).
139. Deirmengian, C., Greenbaum, J., Lotke, P. A., Booth, R. E. & Lonner, J. H. Limited success
     with open debridement and retention of components in the treatment of acute staphylococcus
     aureus infections after total knee arthroplasty. *J. Arthroplasty* **18**, 22–26 (2003).
140. Deirmengian, C. *et al.* Open debridement of acute gram-positive infections after total
     knee arthroplasty. *Clin. Orthop. Relat. Res.* 129–34 (2003). doi:https://doi.org/10.1097/01.
     blo.0000092996.90435.35
141. Horriat, S., Ayyad, S., Thakrar, R. & Haddad, F. Debridement, Antibiotics and Implant
     Retention in Management of Infected Total Knee Arthroplasty; a systematic review. *Semin.
     Arthroplasty* (2019). doi:https://doi.org/10.1053/J.SART.2019.01.012

142. Triantafyllopoulos, G. *et al.* Multiple Irrigation and Debridements for Periprosthetic Joint Infections: Facing a Necessity or Just Prolonging the Inevitable? *J. Arthroplasty* **31**, 219–224 (2016).

143. Haasper, C. & Gehrke, T. Late Infections of the Knee Joint: One-Stage Approach with Cement. in *Periprosthetic Joint Infections Changing Paradigms* (eds. Kendoff, D., Morgan-Jones, R. & Haddad, F. S.) (Springer International Publishing, 2016).

144. von Foerster, G., Klüber, D. & Kähler, U. [Mid- to long-term results after treatment of 118 cases of periprosthetic infections after knee joint replacement using one-stage exchange surgery]. *Orthopade* **20**, 244–52 (1991).

145. Nikolaus, O. B. & Abdel, M. P. Late Infections of the Knee Joint: Two-Stage Articulating Solutions. in *Periprosthetic Joint Infections Changing Paradigms* (eds. Kendoff, D., Morgan-Jones, R. & Haddad, F. S.) (Springer International Publishing, 2016).

146. Fehring, T. K., Odum, S., Calton, T. F. & Mason, J. B. Articulating versus static spacers in revision total knee arthroplasty for sepsis. The Ranawat Award. *Clin. Orthop. Relat. Res.* 9–16 (2000). doi:https://doi.org/10.1097/00003086-200011000-00003

147. McPherson, E. J., Lewonowski, K. & Dorr, L. D. Techniques in Arthroplasty: Use of an articulated PMMA spacer in the infected total knee arthroplasty. *J. Arthroplasty* **10**, 87–89 (1995).

148. Prasad, N., Paringe, V., Kotwal, R., Ghandour, A. & Jones, R. M. Two-stage revision for infected total knee arthroplasty: our experience with interval prosthesis. *Eur. J. Orthop. Surg. Traumatol.* **24**, 1279–1283 (2014).

149. Gooding, C. R., Masri, B. A., Duncan, C. P., Greidanus, N. V. & Garbuz, D. S. Durable infection control and function with the PROSTALAC spacer in two-stage revision for infected knee arthroplasty. in *Clinical Orthopaedics and Related Research* **469**, 985–993 (Springer New York LLC, 2011).

150. Pascale, V. & Pascale, W. Custom-made articulating spacer in two-stage revision total knee arthroplasty. An early follow-up of 14 cases of at least 1 year after surgery. *HSS J.* **3**, 159–163 (2007).

151. Booth, R. E. & Lotke, P. A. The results of spacer block technique in revision of infected total knee arthroplasty. in *Clinical Orthopaedics and Related Research* 57–60 (1989).

152. Borden, L. S. & Gearen, P. F. Infected total knee arthroplasty. A protocol for management. *J. Arthroplasty* **2**, 27–36 (1987).

153. Hofmann, A. A., Kane, K. R., Tkach, T. K., Plaster, R. L. & Camargo, M. P. Treatment of infected total knee arthroplasty using an articulating spacer. *Clin. Orthop. Relat. Res.* 45–54 (1995).

154. Emerson, R. H., Muncie, M., Tarbox, T. R. & Higgins, L. L. Comparison of a static with a mobile spacer in total knee infection. *Clin. Orthop. Relat. Res.* 132–8 (2002). doi:https://doi.org/10.1097/00003086-200211000-00023

155. Guild, G. N., Wu, B. & Scuderi, G. R. Articulating Vs. Static Antibiotic Impregnated Spacers in revision total knee arthroplasty for sepsis. A systematic review. *J. Arthroplasty* **29**, 558–563 (2014).

156. Wilding, C. P., Parry, M. C. & Jeys, L. Late Infections of the Knee Joint: Two-staged Static Solutions. in *Periprosthetic Joint Infections Changing Paradigms* (eds. Kendoff, D., Morgan-Jones, R. & Haddad, F. S.) (Springer International Publishing, 2016).

157. Conway, J. D., Mont, M. A. & Bezwada, H. P. Arthrodesis of the knee. *J. Bone Joint Surg. Am.* **86**, 835–48 (2004).

158. Miralles-Muñoz, F. A., Lizaur-Utrilla, A., Manrique-Lipa, C. & López-Prats, F. A. Artrodesis sin fusión ósea con clavo modular intramedular para revisión de prótesis total de rodilla infectada. *Rev. Esp. Cir. Ortop. Traumatol.* **58**, 217–222 (2014).

159. Kuo, A. C., Meehan, J. P. & Lee, M. Knee fusion using dual platings with the locking compression plate. *J. Arthroplasty* **20**, 772–776 (2005).

160. Raskolnikov, D., Slover, J. D. & Egol, K. A. The use of a multiplanar, multi-axis external fixator to achieve knee arthrodesis in a worst case scenario: a case series. *Iowa Orthop. J.* **33**, 19–24 (2013).

161. Wada, T. *et al.* Resection arthrodesis of the knee with a vascularised fibular graft. *J. Bone Joint Surg. Br.* **82**-**B**, 489–493 (2000).

162. Huang, C. T. *et al.* Amputation: energy cost of ambulation. *Arch. Phys. Med. Rehabil.* **60**, 18–24 (1979).

163. Waters, R. L., Perry, J., Antonelli, D. & Hislop, H. Energy cost of walking of amputees: the influence of level of amputation. *J. Bone Joint Surg. Am.* **58**, 42–6 (1976).

164. Lenguerrand, E. *et al.* Revision for prosthetic joint infection following hip arthroplasty: Evidence from the National Joint Registry. *Bone Jt. Res.* **6**, 391–398 (2017).

165. Muller, M. E. Preservation of septic total hip replacement versus girdlestone operation. in *The Hip: Proceedings of the Second Open Scientific Meeting of The Hip Society* 308 (CV Mosby, St. Louis, 1974).

166. Coventry, M. B. Treatment of infections occurring in total hip surgery. *Orthop. Clin. North Am.* **6**, 991–1003 (1975).

167. Burton, D. S. & Schurman, D. J. Salvage of infected total joint replacements. *Arch. Surg.* **112**, 574–8 (1977).

168. Tsukayama, D. T., Estrada, R. & Gustilo, R. B. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. *J. Bone Joint Surg. Am.* **78**, 512–23 (1996).

169. Brandt, C. M. *et al.* Staphylococcus aureus prosthetic joint infection treated with debridement and prosthesis retention. *Clin. Infect. Dis.* **24**, 914–9 (1997).

170. Brandt, C. M. *et al.* Staphylococcus aureus Prosthetic Joint Infection Treated with Debridement and Prosthesis. *Source Clin. Infect. Dis.* **24**, 914–919 (1997).

171. Drancourt, M. *et al.* Oral rifampin plus ofloxacin for treatment of Staphylococcus-infected orthopedic implants. *Antimicrob. Agents Chemother.* **37**, 1214–8 (1993).

172. Perry, C. R., Hulsey, R. E., Mann, F. A., Miller, G. A. & Pearson, R. L. Treatment of acutely infected arthroplasties with incision, drainage, and local antibiotics delivered via an implantable pump. *Clin. Orthop. Relat. Res.* 216–23 (1992).

173. Widmer, A. F., Gaechter, A., Ochsner, P. E. & Zimmerli, W. Antimicrobial Treatment of Orthopedic Implant-related Infections with Rifampin Combinations. *Clin. Infect. Dis.* (1992). doi:https://doi.org/10.1093/clinids/14.6.1251

174. Zimmerli, W., Widmer, A. F., Blatter, M., Frei, R. & Ochsner, P. E. Role of Rifampin for Treatment of Orthopedic Implant–Related Staphylococcal Infections A Randomized Controlled Trial. *JAMA (Journal Am. Med. Assoc.)* **279**, 1537–1541 (1998).

175. Strange, S. *et al.* One-stage or two-stage revision surgery for prosthetic hip joint infection – the INFORM trial: a study protocol for a randomised controlled trial. *Trials* **17**, 90 (2016).

176. Cahill, J. L., Shadbolt, B., Scarvell, J. M. & Smith, P. N. Quality of life after infection in total joint replacement. *J. Orthop. Surg. (Hong Kong)* **16**, 58–65 (2008).

177. Klouche, S., Sariali, E. & Mamoudy, P. Total hip arthroplasty revision due to infection: A cost analysis approach. *Orthop. Traumatol. Res.* **96**, 124–132 (2010).

178. Del Pozo, J. L. & Patel, R. Infection Associated with Prosthetic Joints. *N. Engl. J. Med.* **361**, 787–794 (2009).

179. Kunutsor, S. K. *et al.* Re-Infection Outcomes following One- and Two-Stage Surgical Revision of Infected Hip Prosthesis: A Systematic Review and Meta-Analysis. *PLoS One* **10**, e0139166 (2015).

180. Garvin, K. L., Konigsberg, B. S. & Hartman, C. W. Late Infections of the Hip Joint: Resection Arthroplasty and Other Solutions. in *Periprosthetic Joint Infections Changing Paradigms* (eds. Kendoff, D., Morgan-Jones, R. & Haddad, F. S.) (Springer International Publishing, 2016).

181. Malcolm, T. L., Gad, B. V., Elsharkawy, K. A. & Higuera, C. A. Complication, Survival, and Reoperation Rates Following Girdlestone Resection Arthroplasty. *J. Arthroplasty* **30**, 1183–1186 (2015).

182. Kralovec, M. E. *et al.* Prosthetic Rehabilitation After Hip Disarticulation or Hemipelvectomy. *Am. J. Phys. Med. Rehabil.* **94**, 1035–1040 (2015).

183. Unruh, T. *et al.* Hip Disarticulation: An 11-Year Experience. *Arch. Surg.* **125**, 791–793 (1990).

184. Gougoulias, N., Khanna, A. & Maffulli, N. How successful are current ankle replacements?: A systematic review of the literature. *Clin. Orthop. Relat. Res.* **468**, 199–208 (2010).
185. Zhou, H., Yakavonis, M., Shaw, J. J., Patel, A. & Li, X. In-patient trends and complications after total ankle arthroplasty in the United States. *Orthopedics* **39**, e74–e79 (2016).
186. Althoff, A., Cancienne, J. M., Cooper, M. T. & Werner, B. C. Patient-Related Risk Factors for Periprosthetic Ankle Joint Infection: An Analysis of 6977 Total Ankle Arthroplasties. *J. Foot Ankle Surg.* **57**, 269–272 (2018).
187. Mazzotti, A. *et al.* Trends in surgical management of the infected total ankle arthroplasty. *Eur. Rev. Med. Pharmacol. Sci.* **23**, 159–172 (2019).
188. Lübbeke, A. *et al.* International variation in shoulder arthroplasty. *Acta Orthop.* **88**, 592–599 (2017).
189. Bohsali, K. I., Wirth, M. A. & Rockwood, C. A. Complications of total shoulder arthroplasty. *J. Bone Joint Surg. Am.* **88**, 2279–92 (2006).
190. Padegimas, E. M. *et al.* Periprosthetic shoulder infection in the United States: incidence and economic burden. *J. Shoulder Elb. Surg.* **24**, 741–746 (2015).
191. Coste, J. S. *et al.* The management of infection in arthroplasty of the shoulder. *J. Bone Joint Surg. Br.* **86**-**B**, 65–69 (2004).
192. Foruria, A. M., Fox, T. J., Sperling, J. W. & Cofield, R. H. Clinical meaning of unexpected positive cultures (UPC) in revision shoulder arthroplasty. *J. Shoulder Elb. Surg.* **22**, 620–627 (2013).
193. Kelly, J. D. & Hobgood, E. R. Positive culture rate in revision shoulder arthroplasty. *Clin. Orthop. Relat. Res.* **467**, 2343–2348 (2009).
194. Mook, W. R., Klement, M. R., Green, C. L., Hazen, K. C. & Garrigues, G. E. The incidence of Propionibacterium acnes in open shoulder surgery: A controlled diagnostic study. *J. Bone Jt. Surg. – Am. Vol.* **97**, 957–963 (2015).
195. Padegimas, E. M. *et al.* Future surgery after revision shoulder arthroplasty: the impact of unexpected positive cultures. *J. Shoulder Elb. Surg.* **26**, 975–981 (2017).
196. Kim, S. J. & Kim, J. H. Unexpected positive cultures including isolation of Propionibacterium acnes in revision shoulder arthroplasty. *Chin. Med. J. (Engl).* **127**, 3975–9 (2014).
197. Levy, O. *et al.* Propionibacterium acnes: An underestimated etiology in the pathogenesis of osteoarthritis? *J. Shoulder Elb. Surg.* **22**, 505–511 (2013).
198. Patel, A., Calfee, R. P., Plante, M., Fischer, S. A. & Green, A. Propionibacterium acnes colonization of the human shoulder. *J. Shoulder Elb. Surg.* **18**, 897–902 (2009).
199. Achermann, Y., Goldstein, E. J. C., Coenye, T. & Shirtliffa, M. E. Propionibacterium acnes: From Commensal to opportunistic biofilm-associated implant pathogen. *Clin. Microbiol. Rev.* **27**, 419–440 (2014).
200. Grosso, M. J., Sabesan, V. J., Ho, J. C., Ricchetti, E. T. & Iannotti, J. P. Reinfection rates after 1-stage revision shoulder arthroplasty for patients with unexpected positive intraoperative cultures. *J. Shoulder Elb. Surg.* **21**, 754–758 (2012).
201. Butler-Wu, S. M. *et al.* Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. *J. Clin. Microbiol.* **49**, 2490–2495 (2011).
202. Updegrove, G. F., Armstrong, A. D. & Kim, H. M. M. Preoperative and intraoperative infection workup in apparently aseptic revision shoulder arthroplasty. *Journal of Shoulder and Elbow Surgery* **24**, 491–500 (2015).
203. Garrigues, G. E. *et al.* Proceedings from the 2018 International Consensus Meeting on Orthopedic Infections: management of periprosthetic shoulder infection. *J. Shoulder Elb. Surg.* **28**, S67–S99 (2019).
204. Stone, G. P. *et al.* Surgical management of periprosthetic shoulder infections. *J. Shoulder Elb. Surg.* **26**, 1222–1229 (2017).
205. Jacquot, A. *et al.* Surgical management of the infected reversed shoulder arthroplasty: A French multicenter study of reoperation in 32 patients. *J. Shoulder Elb. Surg.* **24**, 1713–1722 (2015).

206. Hackett, D. J., Hsu, J. E. & Matsen, F. A. Primary Shoulder Hemiarthroplasty: What Can Be Learned from 359 Cases That Were Surgically Revised? *Clin. Orthop. Relat. Res.* **476**, 1031–1040 (2018).

207. Hernandez, N. M. *et al.* Revision to Reverse Total Shoulder Arthroplasty Restores Stability for Patients With Unstable Shoulder Prostheses. *Clin. Orthop. Relat. Res.* **475**, 2716–2722 (2017).

208. Dines, J. S. *et al.* Outcomes analysis of revision total shoulder replacement. *Journal of Bone and Joint Surgery – Series A* **88**, 1494–1500 (2006).

209. Kany, J. *et al.* The main cause of instability after unconstrained shoulder prosthesis is soft tissue deficiency. *J. Shoulder Elb. Surg.* **26**, e243–e251 (2017).

210. Cuff, D. J., Pupello, D. R., Santoni, B. G., Clark, R. E. & Frankle, M. A. Reverse shoulder arthroplasty for the treatment of rotator cuff deficiency a concise follow-up, at a minimum of 10 years, of previous reports. *J. Bone Jt. Surg. - Am. Vol.* **99**, 1895–1899 (2017).

211. Beekman, P. D. A., Katusic, D., Berghs, B. M., Karelse, A. & De Wilde, L. One-stage revision for patients with a chronically infected reverse total shoulder replacement. *J. Bone Jt. Surg. – Ser. B* **92**, 817–822 (2010).

212. Klatte, T. O. *et al.* Single-stage revision for peri-prosthetic shoulder infection. *Bone Joint J.* **95**-**B**, 391–395 (2013).

213. Sabesan, V. J., Ho, J. C., Kovacevic, D. & Iannotti, J. P. Two-stage reimplantation for treating prosthetic shoulder infections. in *Clinical Orthopaedics and Related Research* **469**, 2538–2543 (Springer New York LLC, 2011).

214. Lee, S. H., Kim, S. J., Kook, S. H. & Kim, J. W. Two-stage revision of infected shoulder arthroplasty using prosthesis of antibiotic-loaded acrylic cement: minimum three-year follow-up. *Int. Orthop.* **42**, 867–874 (2018).

215. Assenmacher, A. T. *et al.* Two-stage reimplantation for the treatment of deep infection after shoulder arthroplasty. *J. Shoulder Elb. Surg.* **26**, 1978–1983 (2017).

216. Hsu, J. E., Gorbaty, J. D., Whitney, I. J. & Matsen, F. A. Single-stage revision is effective for failed shoulder arthroplasty with positive cultures for propionibacterium. *Journal of Bone and Joint Surgery - American Volume* **98**, 2047–2051 (2016).

217. Gamradt, S., Gelber, J. & Zhang, A. Shoulder function and pain level after revision of failed reverse shoulder replacement to hemiarthroplasty. *Int. J. Shoulder Surg.* **6**, 29 (2012).

218. Farshad, M. & Gerber, C. Reverse total shoulder arthroplasty-from the most to the least common complication. *International Orthopaedics* **34**, 1075–1082 (2010).

219. Antuna, S. A., Sperling, J. W., Cofield, R. H. & Rowland, C. M. Glenoid revision surgery after total shoulder arthroplasty. *J. Shoulder Elb. Surg.* **10**, 217–224 (2001).

220. Bonnevialle, N. *et al.* Aseptic glenoid loosening or failure in total shoulder arthroplasty: Revision with glenoid reimplantation. *J. Shoulder Elb. Surg.* **22**, 745–751 (2013).

221. Day, J. S. *et al.* Prevalence and projections of total shoulder and elbow arthroplasty in the United States to 2015. *J. Shoulder Elb. Surg.* **19**, 1115–1120 (2010).

222. Voloshin, I., Schippert, D. W., Kakar, S., Kaye, E. K. & Morrey, B. F. Complications of total elbow replacement: A systematic review. *J. Shoulder Elb. Surg.* **20**, 158–168 (2011).

223. Yamaguchi, K., Adams, R. A. & Morrey, B. F. Infection after Total Elbow Arthroplasty*. *J. Bone Jt. Surg.* **80**, 481–491 (1998).

224. National Joint Registry for England, Wales, N. I. and the I. of M. *14th Annual Report.* (2017).

225. Berbari, E. F. *et al.* Outcome of Prosthetic Joint Infection in Patients with Rheumatoid Arthritis: The Impact of Medical and Surgical Therapy in 200 Episodes. *Clin. Infect. Dis.* **42**, 216–223 (2006).

226. Aldridge, J. M., Lightdale, N. R., Mallon, W. J. & Coonrad, R. W. Total elbow arthroplasty with the Coonrad/Coonrad-Morrey prosthesis. *J. Bone Joint Surg. Br.* **88**-**B**, 509–514 (2006).

227. van der Lugt, J. C. T., Geskus, R. B. & Rozing, P. M. Primary Souter-Strathclyde total elbow prosthesis in rheumatoid arthritis. *J. Bone Joint Surg. Am.* **86**, 465–73 (2004).

228. Sneftrup, S. B., Jensen, S. L., Johannsen, H. V. & Søjbjerg, J. O. Revision of failed total elbow arthroplasty with use of a linked implant. *J. Bone Joint Surg. Br.* **88**-**B**, 78–83 (2006).

229. Rangan, A. *et al.* Investigation and Management of Periprosthetic Joint Infection in the Shoulder and Elbow: Evidence and consensus based guidelines of the British Elbow and Shoulder Society. *Shoulder Elb.* **10**, S5–S19 (2018).

230. Achermann, Y. *et al.* Characteristics and outcome of 27 elbow periprosthetic joint infections: Results from a 14-year cohort study of 358 elbow prostheses. *Clin. Microbiol. Infect.* **17**, 432–438 (2011).

231. BAJIR Bone & Joint Infection Registry – Improving care for patients with Bone and Joint Infections. Available at: https://bajirdotorg.wordpress.com/. (Accessed: 13th September 2019)

232. Ravi, B. *et al.* Relation between surgeon volume and risk of complications after total hip arthroplasty: propensity score matched cohort study. *BMJ* **348**, g3284 (2014).

233. Badawy, M., Espehaug, B., Indrekvam, K., Havelin, L. I. & Furnes, O. Higher revision risk for unicompartmental knee arthroplasty in low-volume hospitals. *Acta Orthop.* **85**, 342–7 (2014).

234. Baker, P. *et al.* Center and surgeon volume influence the revision rate following unicondylar knee replacement: an analysis of 23,400 medial cemented unicondylar knee replacements. *J. Bone Joint Surg. Am.* **95**, 702–9 (2013).

235. Liddle, A. D., Pandit, H., Judge, A. & Murray, D. W. Effect of Surgical Caseload on Revision Rate Following Total and Unicompartmental Knee Replacement. *J. Bone Joint Surg. Am.* **98**, 1–8 (2016).

236. Parvizi, J. *et al.* New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. *Clin. Orthop. Relat. Res.* **469**, 2992–4 (2011).

237. Minassian, A. M., Osmon, D. R. & Berendt, A. R. Clinical guidelines in the management of prosthetic joint infection. *J. Antimicrob. Chemother.* **69**, i29–i35 (2014).

238. Matthews, P. C. *et al.* Outpatient parenteral antimicrobial therapy (OPAT): is it safe for selected patients to self-administer at home? A retrospective analysis of a large cohort over 13 years. *J. Antimicrob. Chemother.* **60**, 356–362 (2007).

239. Yan, C. H. *et al.* Team Approach: The management of infection after total knee replacement. *JBJS Rev.* **6**, e9 (2018).

240. Bucholz, H. W., Elson, R. & Lodenkamper, H. The infected joint implant. in *Recent Advances in orthopedics* (ed. McKibbin, R.) 139–161 (Churchill Livingstone, 1979).

241. Girard, L. P., Ceri, H., Gibb, A. P., Olson, M. & Sepandj, F. MIC Versus MBEC to Determine the Antibiotic Sensitivity of Staphylococcus aureus in Peritoneal Dialysis Peritonitis. *Perit. Dial. Int.* **30**, 652–656 (2010).

242. Olson, M. E., Ceri, H., Morck, D. W., Buret, A. G. & Read, R. R. Biofilm bacteria: Formation and comparative susceptibility to antibiotics. *Can. J. Vet. Res.* **66**, 86–92 (2002).

243. Donlan, R. M. & Costerton, J. W. Biofilms: survival mechanisms of clinically relevant micro-organisms. *Clin. Microbiol. Rev.* **15**, 167–93 (2002).

244. Mah, T. F. C. & O'Toole, G. A. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol.* **9**, 34–39. (2001).

245. Bhattacharya, M., Wozniak, D. J., Stoodley, P. & Hall-Stoodley, L. Prevention and treatment of Staphylococcus aureus biofilms. *Expert Rev. Anti. Infect. Ther.* **13**, 1499 (2015).

246. Anderl, J. N., Zahller, J., Roe, F. & Stewart, P. S. Role of nutrient limitation and stationary-phase existence in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. *Antimicrob. Agents Chemother.* **47**, 1251–6 (2003).

247. Rowe, S. E., Conlon, B. P., Keren, I. & Lewis, K. Persisters: Methods for Isolation and Identifying Contributing Factors—A Review. in *Bacterial Persistence* **1333**, 17–28 (Humana Press, New York, NY, 2016).

248. Bartlett, A. H. & Hulten, K. G. Staphylococcus aureus pathogenesis: secretion systems, adhesins, and invasins. *Pediatr. Infect. Dis. J.* **29**, 860–1 (2010).

249. Otto, M. Basis of Virulence in Community-Associated Methicillin-Resistant *Staphylococcus aureus*. *Annu. Rev. Microbiol.* **64**, 143–162 (2010).

250. Yoong, P. & Torres, V. J. The effects of Staphylococcus aureus leukotoxins on the host: cell lysis and beyond. *Curr. Opin. Microbiol.* **16**, 63–9 (2013).

251. Josse, J., Velard, F. & Gangloff, S. C. Staphylococcus aureus vs. Osteoblast: Relationship and Consequences in Osteomyelitis. *Front. Cell. Infect. Microbiol.* **5**, 85 (2015).

252. Allegranzi, B. *et al.* New WHO recommendations on preoperative measures for surgical site infection prevention: an evidence-based global perspective. *Lancet Infect. Dis.* **11**, 1–12 (2016).

253. Tsang, S. T. J. *et al.* Evaluation of Staphylococcus aureus eradication therapy in orthopaedic surgery. *J. Med. Microbiol.* (2018). doi:https://doi.org/10.1099/jmm.0.000731

254. Bode, L. G. M. *et al.* Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. *N. Engl. J. Med.* **362**, 9–17 (2010).

255. Schweizer, M. L. *et al.* Association of a Bundled Intervention With Surgical Site Infections Among Patients Undergoing Cardiac, Hip, or Knee Surgery. *JAMA* **313**, 2162 (2015).

256. Maslow, J. *et al.* Patient experience with mupirocin or povidone-iodine nasal decolonization. *Orthopedics* **37**, e576–81 (2014).

257. Hudson, I. R. B. The efficacy of intranasal mupirocin in the prevention of staphylococcal infections: a review of recent experience. *J. Hosp. Infect.* **27**, 81–98 (1994).

258. Caffrey, A. R., Quilliam, B. J. & LaPlante, K. L. Risk factors associated with mupirocin resistance in meticillin-resistant Staphylococcus aureus. *J. Hosp. Infect.* **76**, 206–210 (2010).

259. Ammerlaan, H. S. M., Kluytmans, J. A. J. W., Wertheim, H. F. L., Nouwen, J. L. & Bonten, M. J. M. Eradication of methicillin-resistant Staphylococcus aureus carriage: a systematic review. *Clin. Infect. Dis.* **48**, 922–30 (2009).

260. Hetem, D. J. & Bonten, M. J. M. Clinical relevance of mupirocin resistance in Staphylococcus aureus. *J. Hosp. Infect.* **85**, 249–56 (2013).

261. Bryce, E. *et al.* Nasal photodisinfection and chlorhexidine wipes decrease surgical site infections: A historical control study and propensity analysis. *J. Hosp. Infect.* **88**, 89–95 (2014).

262. Bornstein, E., Hermans, W., Gridley, S. & Manni, J. Near-infrared photoinactivation of bacteria and fungi at physiologic temperatures. *Photochem. Photobiol.* **85**, 1364–74 (2009).

263. Gwynne, P. J. & Gallagher, M. P. Light as a Broad-Spectrum Antimicrobial. *Front. Microbiol.* **9**, 119 (2018).

264. Maclean, M. *et al.* Continuous decontamination of an intensive care isolation room during patient occupancy using 405 nm light technology. *J. Infect. Prev.* **14**, 176–181 (2013).

265. Hook, A. L. *et al.* Combinatorial discovery of polymers resistant to bacterial attachment. *Nat. Biotechnol.* **30**, 868–875 (2012).

266. Kucharíková, S. *et al.* Covalent immobilization of antimicrobial agents on titanium prevents *Staphylococcus aureus* and *Candida albicans* colonization and biofilm formation. *J. Antimicrob. Chemother.* **71**, 936–945 (2016).

267. Knetsch, M. L. W. & Koole, L. H. New Strategies in the Development of Antimicrobial Coatings: The Example of Increasing Usage of Silver and Silver Nanoparticles. *Polymers (Basel).* **3**, 340–366 (2011).

268. Alt, V. Antimicrobial coated implants in trauma and orthopaedics–A clinical review and risk-benefit analysis. *Injury* **48**, 599–607 (2017).

269. Jaggessar, A., Shahali, H., Mathew, A. & Yarlagadda, P. K. D. V. Bio-mimicking nano and micro-structured surface fabrication for antibacterial properties in medical implants. *J. Nanobiotechnology* **15**, 64 (2017).

270. Palmer, J., Flint, S. & Brooks, J. Bacterial cell attachment, the beginning of a biofilm. *J. Ind. Microbiol. Biotechnol.* **34**, 577–588 (2007).

271. Renner, L. D. & Weibel, D. B. Physicochemical regulation of biofilm formation. *MRS Bull.* **36**, 347–355 (2011).

272. Helbig, R. *et al.* The impact of structure dimensions on initial bacterial adhesion. *Biomater. Sci.* **4**, 1074–1078 (2016).

273. Ostuni, E. *et al.* Self-Assembled Monolayers That Resist the Adsorption of Proteins and the Adhesion of Bacterial and Mammalian Cells. *Langmuir* **17**, 6336–6343 (2001).

274. Liu, Y., Strauss, J. & Camesano, T. A. Thermodynamic Investigation of Staphylococcus epidermidis Interactions with Protein-Coated Substrata. *Langmuir* **23**, 7134–7142 (2007).

275. An, Y. H. & Friedman, R. J. Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. *J. Biomed. Mater. Res.* **43**, 338–348 (1998).

276. Bruzaud, J. *et al.* The design of superhydrophobic stainless steel surfaces by controlling nanostructures: A key parameter to reduce the implantation of pathogenic bacteria. *Mater. Sci. Eng. C* **73**, 40–47 (2017).

277. Hizal, F. *et al.* Nanoengineered Superhydrophobic Surfaces of Aluminum with Extremely Low Bacterial Adhesivity. *ACS Appl. Mater. Interfaces* **9**, 12118–12129 (2017).

278. Ren, Y. *et al.* Emergent heterogeneous microenvironments in biofilms: substratum surface heterogeneity and bacterial adhesion force-sensing. *FEMS Microbiol. Rev.* **42**, 259–272 (2018).

279. James, S. A., Hilal, N. & Wright, C. J. Atomic force microscopy studies of bioprocess engineering surfaces - imaging, interactions and mechanical properties mediating bacterial adhesion. *Biotechnol. J.* **12**, 1600698 (2017).

280. Ivanova, E. P. *et al.* Differential attraction and repulsion of Staphylococcus aureus and Pseudomonas aeruginosa on molecularly smooth titanium films. *Sci. Rep.* **1**, 165 (2011).

281. Bagherifard, S. *et al.* The influence of nanostructured features on bacterial adhesion and bone cell functions on severely shot peened 316L stainless steel. *Biomaterials* **73**, 185–197 (2015).

282. Ista, L. K., Fan, H., Baca, O. & López, G. P. Attachment of bacteria to model solid surfaces: oligo(ethylene glycol) surfaces inhibit bacterial attachment. *FEMS Microbiol. Lett.* **142**, 59–63 (1996).

283. Smith, R. S. *et al.* Vascular catheters with a nonleaching poly-sulfobetaine surface modification reduce thrombus formation and microbial attachment. *Sci. Transl. Med.* **4**, 153ra132 (2012).

284. Chauhan, A. *et al.* Preventing Biofilm Formation and Associated Occlusion by Biomimetic Glycocalyxlike Polymer in Central Venous Catheters. *J. Infect. Dis.* **210**, 1347–1356 (2014).

285. Hwang, I. -s., Hwang, J. H., Choi, H., Kim, K.-J. & Lee, D. G. Synergistic effects between silver nanoparticles and antibiotics and the mechanisms involved. *J. Med. Microbiol.* **61**, 1719–1726 (2012).

286. Brennan, S. A. *et al.* Silver nanoparticles and their orthopaedic applications. *Bone Joint J.* **97-B**, 582–589 (2015).

287. Wafa, H. *et al.* Retrospective evaluation of the incidence of early periprosthetic infection with silver-treated endoprostheses in high-risk patients. *Bone Joint J.* **97**-**B**, 252–257 (2015).

288. Kollef, M. H. *et al.* Silver-coated endotracheal tubes and incidence of ventilator-associated pneumonia: The NASCENT randomized trial. *JAMA – J. Am. Med. Assoc.* (2008). doi:https://doi.org/10.1001/jama.300.7.805

289. Schierholz, J. M., Lucas, L. J., Rump, A. & Pulverer, G. Efficacy of silver-coated medical devices. *J. Hosp. Infect.* **40**, 257–262 (1998).

290. van Hengel, I. A. J. *et al.* Selective laser melting porous metallic implants with immobilized silver nanoparticles kill and prevent biofilm formation by methicillin-resistant Staphylococcus aureus. *Biomaterials* **140**, 1–15 (2017).

291. Gao, L. *et al.* Nanocatalysts promote Streptococcus mutans biofilm matrix degradation and enhance bacterial killing to suppress dental caries in vivo. *Biomaterials* **101**, 272–84 (2016).

292. Jennings, J. A. *et al.* Novel Antibiotic-loaded Point-of-care Implant Coating Inhibits Biofilm. *Clin. Orthop. Relat. Res.* **473**, 2270–2282 (2015).

293. Giavaresi, G. *et al.* Efficacy of antibacterial-loaded coating in an in vivo model of acutely highly contaminated implant. *Int. Orthop.* **38**, 1505–1512 (2014).

294. Drago, L. *et al.* Does Implant Coating With Antibacterial-Loaded Hydrogel Reduce Bacterial Colonization and Biofilm Formation in Vitro? *Clin. Orthop. Relat. Res.* **472**, 3311 (2014).

295. Romanò, C. L., Tsuchiya, H., Morelli, I., Battaglia, A. G. & Drago, L. Antibacterial coating of implants: are we missing something? *Bone Jt. Res.* **8**, 199–206 (2019).

296. Romanò, C. L. *et al.* Does an Antibiotic-Loaded Hydrogel Coating Reduce Early Post-Surgical Infection After Joint Arthroplasty? *J. bone Jt. Infect.* **1**, 34–41 (2016).

297. Schütz, C. A., Juillerat-Jeanneret, L., Mueller, H., Lynch, I. & Riediker, M. Therapeutic nanoparticles in clinics and under clinical evaluation. *Nanomedicine* **8**, 449–467 (2013).

298. Rukavina, Z. & Vanić, Ž. Current Trends in Development of Liposomes for Targeting Bacterial Biofilms. *Pharmaceutics* **8**, (2016).

299. Forier, K. *et al.* Lipid and polymer nanoparticles for drug delivery to bacterial biofilms. *J. Control. Release* **190**, 607–623 (2014).

300. Zazo, H., Colino, C. I. & Lanao, J. M. Current applications of nanoparticles in infectious diseases. *J. Control. Release* **224**, 86–102 (2016).

301. Liu, Y. *et al.* Surface-Adaptive, Antimicrobially Loaded, Micellar Nanocarriers with Enhanced Penetration and Killing Efficiency in Staphylococcal Biofilms. *ACS Nano* **10**, 4779–4789 (2016).

302. Radovic-Moreno, A. F. *et al.* Surface charge-switching polymeric nanoparticles for bacterial cell wall-targeted delivery of antibiotics. *ACS Nano* **6**, 4279–87 (2012).

303. Koo, H., Allan, R. N., Howlin, R. P., Stoodley, P. & Hall-Stoodley, L. Targeting microbial biofilms: current and prospective therapeutic strategies. *Nat. Rev. Microbiol.* **15**, 740–755 (2017).

304. Busscher, H. J. *et al.* Biomaterial-associated infection: Locating the finish line in the race for the surface. *Science Translational Medicine* (2012). doi:https://doi.org/10.1126/scitranslmed.3004528

305. Hasan, J., Crawford, R. J. & Ivanova, E. P. Antibacterial surfaces: the quest for a new generation of biomaterials. *Trends Biotechnol.* **31**, 295–304 (2013).

306. Campoccia, D., Montanaro, L. & Arciola, C. R. A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces. *Biomaterials* **34**, 8018–8029 (2013).

307. Muszanska, A. K. *et al.* Antiadhesive Polymer Brush Coating Functionalized with Antimicrobial and RGD Peptides to Reduce Biofilm Formation and Enhance Tissue Integration. *Biomacromolecules* **15**, 2019–2026 (2014).

308. Nguyen, D. *et al.* Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science* **334**, 982–6 (2011).

309. Amato, S. M. *et al.* The role of metabolism in bacterial persistence. *Front. Microbiol.* **5**, 70 (2014).

310. de la Fuente-Núñez, C., Reffuveille, F., Haney, E. F., Straus, S. K. & Hancock, R. E. W. Broad-Spectrum Anti-biofilm Peptide That Targets a Cellular Stress Response. *PLoS Pathog.* (2014). doi:https://doi.org/10.1371/journal.ppat.1004152

311. Reffuveille, F., de la Fuente-Núñez, C., Mansour, S. & Hancock, R. E. W. A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms. *Antimicrob. Agents Chemother.* **58**, 5363–71 (2014).

312. Weidenmaier, C. *et al.* DltABCD- and MprF-Mediated Cell Envelope Modifications of Staphylococcus aureus Confer Resistance to Platelet Microbicidal Proteins and Contribute to Virulence in a Rabbit Endocarditis Model. *Infect. Immun.* **73**, 8033–8038 (2005).

313. Allison, K. R., Brynildsen, M. P. & Collins, J. J. Metabolite-enabled eradication of bacterial persisters by aminoglycosides. *Nature* **473**, 216–220 (2011).

314. Barraud, N., Buson, A., Jarolimek, W. & Rice, S. A. Mannitol Enhances Antibiotic Sensitivity of Persister Bacteria in Pseudomonas aeruginosa Biofilms. *PLoS One* **8**, e84220 (2013).

315. Lebeaux, D. *et al.* pH-Mediated Potentiation of Aminoglycosides Kills Bacterial Persisters and Eradicates In Vivo Biofilms. *J. Infect. Dis.* **210**, 1357–1366 (2014).

316. Prax, M., Mechler, L., Weidenmaier, C. & Bertram, R. Glucose Augments Killing Efficiency of Daptomycin Challenged Staphylococcus aureus Persisters. *PLoS One* **11**, e0150907 (2016).

317. Lebeaux, D., Ghigo, J.-M. & Beloin, C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiol. Mol. Biol. Rev.* **78**, 510–43 (2014).

318. Jørgensen, N. P. *et al.* Rifampicin-containing combinations are superior to combinations of vancomycin, linezolid and daptomycin against *Staphylococcus aureus* biofilm infection *in vivo* and *in vitro*. *Pathog. Dis.* **74**, ftw019 (2016).

319. Niska, J. A. *et al.* Vancomycin-rifampin combination therapy has enhanced efficacy against an experimental Staphylococcus aureus prosthetic joint infection. *Antimicrob. Agents Chemother.* **57**, 5080–6 (2013).

320. Olson, M. E., Slater, S. R., Rupp, M. E. & Fey, P. D. Rifampicin enhances activity of dapto-mycin and vancomycin against both a polysaccharide intercellular adhesin (PIA)-dependent and -independent Staphylococcus epidermidis biofilm. *J. Antimicrob. Chemother.* **65**, 2164–2171 (2010).

321. Bollenbach, T., Quan, S., Chait, R. & Kishony, R. Nonoptimal microbial response to antibiot-ics underlies suppressive drug interactions. *Cell* **139**, 707–18 (2009).

322. Chait, R., Craney, A. & Kishony, R. Antibiotic interactions that select against resistance. *Nature* **446**, 668–671 (2007).

323. Yeh, P., Tschumi, A. I. & Kishony, R. Functional classification of drugs by properties of their pairwise interactions. *Nat. Genet.* **38**, 489–494 (2006).

324. Beppler, C. *et al.* When more is less: Emergent suppressive interactions in three-drug combi-nations. *BMC Microbiol.* **17**, 1–9 (2017).

325. Weidenmaier, C. & Lee, J. C. Structure and Function of Surface Polysaccharides of Staphylococcus aureus. in *Staphylococcus aureus* 57–93 (Springer, Cham, 2015). doi:https://doi.org/10.1007/82_2015_5018

326. Yang, S.-J. S.-J. *et al.* The Staphylococcus aureus Two-Component Regulatory System, GraRS, Senses and Confers Resistance to Selected Cationic Antimicrobial Peptides. *Infect. Immun.* **80**, 74–81 (2012).

327. Kohanski, M. A., Dwyer, D. J. & Collins, J. J. How antibiotics kill bacteria: from targets to networks. *Nat. Publ. Gr.* **8**, 423–435 (2010).

328. Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A. & Collins, J. J. A Common Mechanism of Cellular Death Induced by Bactericidal Antibiotics. *Cell* **130**, 797–810 (2007).

329. Kelley, W. L., Lew, D. P. & Renzoni, A. Antimicrobial Peptide Exposure and Reduced Susceptibility to Daptomycin: Insights Into a Complex Genetic Puzzle. *J. Infect. Dis.* **206**, 1153–1156 (2012).

330. Cui, L., Lian, J.-Q., Neoh, H.-M., Reyes, E. & Hiramatsu, K. DNA microarray-based identification of genes associated with glycopeptide resistance in Staphylococcus aureus. *Antimicrob. Agents Chemother.* **49**, 3404–13 (2005).

331. Lewis, K. Persister cells and the riddle of biofilm survival. *Biochemistry-Moscow* **70**, 267-+ (2005).

332. Boles, B. R. & Horswill, A. R. agr-Mediated Dispersal of Staphylococcus aureus Biofilms. *PLoS Pathog.* **4**, e1000052 (2008).

333. França, A., Carvalhais, V., Vilanova, M., Pier, G. B. & Cerca, N. Characterization of an in vitro fed-batch model to obtain cells released from S. epidermidis biofilms. *AMB Express* **6**, 23 (2016).

334. Conlon, B. P. *et al.* Activated ClpP kills persisters and eradicates a chronic biofilm infection. *Nature* **503**, 365–70 (2013).

335. Conlon, B. P., Rowe, S. E. & Lewis, K. Persister Cells in Biofilm Associated Infections. in *Advances in experimental medicine and biology* **831**, 1–9 (Springer, Cham, 2015).

336. Ling, L. L. *et al.* A new antibiotic kills pathogens without detectable resistance. *Nature* **517**, 455–459 (2015).

337. Conlon, B. P. *et al.* Persister formation in Staphylococcus aureus is associated with ATP depletion. *Nat. Microbiol.* **1**, 16051 (2016).

338. Peacock, S. J. & Paterson, G. K. Mechanisms of Methicillin Resistance in *Staphylococcus aureus.* *Annu. Rev. Biochem.* **84**, 577–601 (2015).

339. Zapotoczna, M., O'Neill, E. & O'Gara, J. P. Untangling the Diverse and Redundant Mechanisms of Staphylococcus aureus Biofilm Formation. *PLoS Pathog.* **12**, e1005671 (2016).

340. O'Gara, J. P. *ica* and beyond: biofilm mechanisms and regulation in *Staphylococcus epider-midis* and *Staphylococcus aureus. FEMS Microbiol. Lett.* **270**, 179–188 (2007).

341. McCarthy, H. *et al.* Methicillin resistance and the biofilm phenotype in Staphylococcus aureus. *Front. Cell. Infect. Microbiol.* **5**, 1 (2015).

342. Waters, E. M. *et al.* Convergence of Staphylococcus aureus Persister and Biofilm Research: Can Biofilms Be Defined as Communities of Adherent Persister Cells? *PLOS Pathog.* **12**, e1006012 (2016).

343. Königs, A. M., Flemming, H.-C. & Wingender, J. Nanosilver induces a non-culturable but metabolically active state in Pseudomonas aeruginosa. *Front. Microbiol.* **06**, 395 (2015).

344. Boles, B. R. & Horswill, A. R. Staphylococcal biofilm disassembly. *Trends Microbiol.* **19**, (2011).

345. Hogan, S., O'Gara, J. P. & O'Neill, E. Novel Treatment of Staphylococcus aureus Device-Related Infections Using Fibrinolytic Agents. *Antimicrob. Agents Chemother.* **62**, e02008–17 (2018).

346. Hogan, S. *et al.* Potential use of targeted enzymatic agents in the treatment of Staphylococcus aureus biofilm-related infections. *J. Hosp. Infect.* **96**, 177–182 (2017).

347. Ricciardi, B. F. *et al.* Staphylococcus aureus Evasion of Host Immunity in the Setting of Prosthetic Joint Infection: Biofilm and Beyond. *Curr. Rev. Musculoskelet. Med.* 1–12 (2018). doi:https://doi.org/10.1007/s12178-018-9501-4

348. Estellés, A. *et al.* A high-affinity native human antibody disrupts biofilm from Staphylococcus aureus bacteria and potentiates antibiotic efficacy in a mouse implant infection model. *Antimicrob. Agents Chemother.* (2016). doi:https://doi.org/10.1128/AAC.02588-15

349. Wang, Y. *et al.* Mouse model of hematogenous implant-related Staphylococcus aureus biofilm infection reveals therapeutic targets. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E5094–E5102 (2017).

350. Pitt, W. G., McBride, M. O., Lunceford, J. K., Roper, R. J. & Sagers, R. D. Ultrasonic enhancement of antibiotic action on gram-negative bacteria. *Antimicrob. Agents Chemother.* **38**, 2577–82 (1994).

351. Del Pozo, J. L. *et al.* The electricidal effect is active in an experimental model of Staphylococcus epidermidis chronic foreign body osteomyelitis. *Antimicrob. Agents Chemother.* **53**, 4064–8 (2009).

352. Del Pozo, J. L., Rouse, M. S. & Patel, R. Bioelectric effect and bacterial biofilms. A systematic review. *Int. J. Artif. Organs* **31**, 786–795 (2008).

353. del Pozo, J. L., Rouse, M. S., Mandrekar, J. N., Steckelberg, J. M. & Patel, R. The electricidal effect: reduction of Staphylococcus and pseudomonas biofilms by prolonged exposure to low-intensity electrical current. *Antimicrob. Agents Chemother.* **53**, 41–5 (2009).

354. Pickering, S.a W. , Bayston, R. & Scammell, B. E. Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants. *J. Bone Jt. Surgery-British Vol.* **85B**, 588–593 (2003).

355. Lauderdale, K. J., Malone, C. L., Boles, B. R., Morcuende, J. & Horswill, A. R. Biofilm dispersal of community-associated methicillin-resistant *Staphylococcus aureus* on orthopedic implant material. *J. Orthop. Res.* **28**, n/a-n/a (2009).

356. Darouiche, R. O., Mansouri, M. D., Gawande, P. V. & Madhyastha, S. Antimicrobial and antibiofilm efficacy of triclosan and DispersinB(R) combination. *J. Antimicrob. Chemother.* **64**, 88–93 (2009).

357. Izano, E. A., Wang, H., Ragunath, C., Ramasubbu, N. & Kaplan, J. B. Detachment and Killing of *Aggregatibacter actinomycetemcomitans* Biofilms by Dispersin B and SDS. *J. Dent. Res.* **86**, 618–622 (2007).

358. Eckhart, L., Fischer, H., Barken, K. B., Tolker-Nielsen, T. & Tschachler, E. DNase1L2 suppresses biofilm formation by Pseudomonas aeruginosa and Staphylococcus aureus. *Br. J. Dermatol.* **156**, 1342–1345 (2007).

359. Kalpana, B. J., Aarthy, S. & Pandian, S. K. Antibiofilm Activity of α-Amylase from Bacillus subtilis S8-18 Against Biofilm Forming Human Bacterial Pathogens. *Appl. Biochem. Biotechnol.* **167**, 1778–1794 (2012).

360. Whitchurch, C. B., Tolker-Nielsen, T., Ragas, P. C. & Mattick, J. S. Extracellular DNA Required for Bacterial Biofilm Formation. *Science (80-.).* **295**, 1487–1487 (2002).

361. Kokai-Kun, J. F., Walsh, S. M., Chanturiya, T. & Mond, J. J. Lysostaphin cream eradicates Staphylococcus aureus nasal colonization in a cotton rat model. *Antimicrob. Agents Chemother.* **47**, 1589–97 (2003).

362. Donelli, G. *et al.* Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. *Antimicrob. Agents Chemother.* **51**, 2733–40 (2007).

363. Kaplan, J. B. *et al.* Recombinant human DNase I decreases biofilm and increases antimicrobial susceptibility in staphylococci. *J. Antibiot. (Tokyo).* **65**, 73–77 (2012).

364. Tetz, G. V, Artemenko, N. K. & Tetz, V. V. Effect of DNase and antibiotics on biofilm characteristics. *Antimicrob. Agents Chemother.* **53**, 1204–9 (2009).

365. Jørgensen, N. *et al.* Streptokinase Treatment Reverses Biofilm-Associated Antibiotic Resistance in Staphylococcus aureus. *Microorganisms* **4**, 36 (2016).

366. Sugai, M. *et al.* Purification and molecular characterization of glycylglycine endopeptidase produced by Staphylococcus capitis EPK1. *J. Bacteriol.* **179**, 1193–202 (1997).

367. Kokai-Kun, J. F., Chanturiya, T. & Mond, J. J. Lysostaphin eradicates established Staphylococcus aureus biofilms in jugular vein catheterized mice. *J. Antimicrob. Chemother.* **64**, 94–100 (2009).

368. Aguinaga, A. *et al.* Lysostaphin and clarithromycin: a promising combination for the eradication of Staphylococcus aureus biofilms. *Int. J. Antimicrob. Agents* **37**, 585–587 (2011).

369. Algburi, A., Comito, N., Kashtanov, D., Dicks, L. M. T. & Chikindas, M. L. Control of Biofilm Formation: Antibiotics and Beyond. *Appl. Environ. Microbiol.* **83**, e02508–16 (2017).

370. European Commission. *Directive 2001/104/EC of the European Parliament and of the Council of 7 December 2001 amending Council Directive 93/42/EEC concerning medical devices*. (2001).

371. Ernest, E. P., Machi, A. S., Karolcik, B. A., LaSala, P. R. & Dietz, M. J. Topical adjuvants incompletely remove adherent Staphylococcus aureus from implant materials. *J. Orthop. Res.* (2017). doi:https://doi.org/10.1002/JOR.23804

372. Johnston, C. S. & Gaas, C. A. Vinegar: medicinal uses and antiglycemic effect. *MedGenMed* **8**, 61 (2006).

373. Williams, N. M. A., Wales, S. & Carlson, G. L. Pseudomonas infection of the catheter exit site successfully managed with topical acetic acid. *Clin. Nutr.* **12**, 369–370 (1993).

374. Hirshfield, I. N., Terzulli, S. & O'Byrne, C. Weak organic acids: a panoply of effects on bacteria. *Sci. Prog.* **86**, 245–69 (2003).

375. Bjarnsholt, T. *et al.* Antibiofilm Properties of Acetic Acid. *Adv. Wound Care* **4**, 363 (2015).

376. Halstead, F. D. *et al.* The Antibacterial Activity of Acetic Acid against Biofilm-Producing Pathogens of Relevance to Burns Patients. *PLoS One* **10**, e0136190 (2015).

377. Kothari, A. Treatment of 'resistant' ottorhoea with acetic acid. *Laryngoscope* **79**, 494–498 (1969).

378. Nagoba, B. S., Selkar, S. P., Wadher, B. J. & Gandhi, R. C. Acetic acid treatment of pseudomonal wound infections – A review. *J. Infect. Public Health* **6**, 410–415 (2013).

379. Leary, J. T. *et al.* Complete Eradication of Biofilm From Orthopedic Materials. *J. Arthroplasty* (2017). doi:https://doi.org/10.1016/j.arth.2017.03.050

380. Ricker, E. B. & Nuxoll, E. Synergistic effects of heat and antibiotics on Pseudomonas aeruginosa biofilms. *Biofouling* **33**, 855–866 (2017).

381. Cheng, D. K. *Field and wave electromagnetics by David K cheng, 2nd Edition*. (1989).

382. Chopra, R. *et al.* Employing high-frequency alternating magnetic fields for the non-invasive treatment of prosthetic joint infections. *Sci. Rep.* **7**, 7520 (2017).

383. Pijls, B. G., Sanders, I. M. J. G., Kuijper, E. J. & Nelissen, R. G. H. H. Non-contact electromagnetic induction heating for eradicating bacteria and yeasts on biomaterials and possible relevance to orthopaedic implant infections. *Bone Jt. Res.* **6**, (2017).

384. Fang, C.-H. *et al.* Magnetic hyperthermia enhance the treatment efficacy of peri-implant osteomyelitis. *BMC Infect. Dis.* **17**, (2017).

385. Pijls, B. G. *et al.* Segmental induction heating of orthopaedic metal implants. *Bone Jt. Res.* **7**, 609–619 (2018).

386. Ehrensberger, M. T. *et al.* Cathodic voltage-controlled electrical stimulation of titanium implants as treatment for methicillin-resistant Staphylococcus aureus periprosthetic infections. *Biomaterials* **41**, 97–105 (2015).

387. Costerton, J. W., Ellis, B., Lam, K., Johnson, F. & Khoury, A. E. Mechanism of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. *Antimicrob. Agents Chemother.* **38**, 2803–9 (1994).

388. Harvey, E. N. & Loomis, A. L. THE DESTRUCTION OF LUMINOUS BACTERIA BY HIGH FREQUENCY SOUND WAVES. *J. Bacteriol.* **17**, 373–6 (1929).

389. Earnshaw, R. G., Appleyard, J. & Hurst, R. . M. Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure. *Int. J. Food Microbiol.* **28**, 197–219 (1995).

390. Butz, P. & Tauscher, B. Emerging technologies: chemical aspects. *Food Res. Int.* **35**, 279–284 (2002).

391. Piyasena, P., Mohareb, E. & McKellar, R. . Inactivation of microbes using ultrasound: a review. *Int. J. Food Microbiol.* **87**, 207–216 (2003).

392. Phull, S., Newman, A., Lorimer, J. & Mason, T. The development and evaluation of ultrasound in the biocidal treatment of water. *Ultrason. Sonochem.* **4**, 157–164 (1997).

393. Erriu, M. *et al.* Microbial biofilm modulation by ultrasound: Current concepts and controversies. *Ultrason. Sonochem.* **21**, 15–22 (2014).

394. Nicholson, J. A., Tsang, S. T. J., MacGillivray, T. J., Perks, F. & Simpson, A. H. R. W. What is the role of ultrasound in fracture management? *Bone Jt. Res.* **8**, 304–312 (2019).

395. Carmen, J. C. *et al.* Ultrasonically enhanced vancomycin activity against Staphylococcus epidermidis biofilms in vivo. *J. Biomater. Appl.* **18**, 237–45 (2004).

396. Wendling, A., Mar, D., Wischmeier, N., Anderson, D. & McIff, T. Combination of modified mixing technique and low frequency ultrasound to control the elution profile of vancomycin-loaded acrylic bone cement. *Bone Joint Res.* **5**, 26–32 (2016).

397. Ensing, G. T. T. *et al.* Effect of pulsed ultrasound in combination with gentamicin on bacterial killing of biofilms on bone cements in vivo. *J. Appl. Microbiol.* **99**, (2005).

398. Ensing, G. T. *et al.* The combination of ultrasound with antibiotics released from bone cement decreases the viability of planktonic and biofilm bacteria: an in vitro study with clinical strains. *J. Antimicrob. Chemother.* **58**, (2006).

399. Bigelow, T. A., Northagen, T., Hill, T. M. & Sailer, F. C. The Destruction of Escherichia Coli Biofilms Using High-Intensity Focused Ultrasound. *Ultrasound Med. Biol.* (2009). doi:https://doi.org/10.1016/j.ultrasmedbio.2008.12.001

400. Ryder, C., Byrd, M. & Wozniak, D. J. Role of polysaccharides in Pseudomonas aeruginosa biofilm development. *Curr. Opin. Microbiol.* **10**, 644–8 (2007).

401. Uroz, S., Dessaux, Y. & Oger, P. Quorum Sensing and Quorum Quenching: The Yin and Yang of Bacterial Communication. *ChemBioChem* **10**, 205–216 (2009).

402. Chen, F. *et al.* Quorum Quenching Enzymes and Their Application in Degrading Signal Molecules to Block Quorum Sensing-Dependent Infection. *Int. J. Mol. Sci.* **14**, 17477–17500 (2013).

403. Francolini, I., Norris, P., Piozzi, A., Donelli, G. & Stoodley, P. Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. *Antimicrob. Agents Chemother.* **48**, 4360–5 (2004).

404. Balaban, N. *et al.* Treatment of Staphylococcus aureus biofilm infection by the quorum-sensing inhibitor RIP. *Antimicrob. Agents Chemother.* **51**, 2226–9 (2007).

405. O'Loughlin, C. T. *et al.* A quorum-sensing inhibitor blocks Pseudomonas aeruginosa virulence and biofilm formation. *Proc. Natl. Acad. Sci.* (2013). doi:https://doi.org/10.1073/pnas.1316981110

406. Brackman, G., Cos, P., Maes, L., Nelis, H. J. & Coenye, T. Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. *Antimicrob. Agents Chemother.* **55**, 2655–61 (2011).

407. Brackman, G. & Coenye, T. Inhibition of Quorum Sensing in Staphylococcus spp. *Curr. Pharm. Des.* **21**, 2101–2108 (2015).

408. Rossi, L. M., Rangasamy, P., Zhang, J., Qiu, X. & Wu, G. Y. Research advances in the development of peptide antibiotics. *J. Pharm. Sci.* **97**, 1060–1070 (2008).

409. Melo, M. N., Ferre, R. & Castanho, M. A. R. B. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nat. Rev. Microbiol.* **7**, 245–250 (2009).

410. Li, L. *et al.* Targeted Antimicrobial Therapy Against Streptococcus mutans Establishes Protective Non-cariogenic Oral Biofilms and Reduces Subsequent Infection. *Int. J. Oral Sci.* **2**, 66–73 (2010).

411. He, J., Anderson, M. H., Shi, W. & Eckert, R. Design and activity of a 'dual-targeted' antimicrobial peptide. *Int. J. Antimicrob. Agents* **33**, 532–537 (2009).

412. Wimley, W. C. & Hristova, K. Antimicrobial Peptides: Successes, Challenges and Unanswered Questions. *J. Membr. Biol.* **239**, 27–34 (2011).

413. Jorge, P., Lourenço, A. & Pereira, M. O. New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. *Biofouling* **28**, 1033–1061 (2012).

414. Beloin, C., Renard, S., Ghigo, J.-M. & Lebeaux, D. Novel approaches to combat bacterial biofilms. *Curr. Opin. Pharmacol.* **18**, 61–68 (2014).

415. Das, T., Sharma, P. K., Busscher, H. J., van der Mei, H. C. & Krom, B. P. Role of extracellular DNA in initial bacterial adhesion and surface aggregation. *Appl. Environ. Microbiol.* **76**, 3405–8 (2010).

416. Fox, J. L. Antimicrobial peptides stage a comeback. *Nat. Biotechnol.* **31**, 379–382 (2013).

417. Tiwari, S. K., Noll, K. S., Cavera, V. L. & Chikindas, M. L. Improved Antimicrobial Activities of Synthetic-Hybrid Bacteriocins Designed from Enterocin E50-52 and Pediocin PA-1. *Appl. Environ. Microbiol.* **81**, 1661–1667 (2015).

418. Ma, L. *et al.* Effects of 14-Alpha-Lipoyl Andrographolide on Quorum Sensing in Pseudomonas aeruginosa. *Antimicrob. Agents Chemother.* **56**, 6088–6094 (2012).

419. Xu, W., Zhu, X., Tan, T., Li, W. & Shan, A. Design of Embedded-Hybrid Antimicrobial Peptides with Enhanced Cell Selectivity and Anti-Biofilm Activity. *PLoS One* **9**, e98935 (2014).

420. Di Luca, M., Maccari, G., Maisetta, G. & Batoni, G. BaAMPs: the database of biofilm-active antimicrobial peptides. *Biofouling* **31**, 193–199 (2015).

421. Altman, H. *et al.* In vitro assessment of antimicrobial peptides as potential agents against several oral bacteria. *J. Antimicrob. Chemother.* **58**, 198–201 (2006).

422. Herbert, S. *et al.* Molecular Basis of Resistance to Muramidase and Cationic Antimicrobial Peptide Activity of Lysozyme in Staphylococci. *PLoS Pathog.* **3**, e102 (2007).

423. Mataraci, E. & Dosler, S. In vitro activities of antibiotics and antimicrobial cationic peptides alone and in combination against methicillin-resistant Staphylococcus aureus biofilms. *Antimicrob. Agents Chemother.* **56**, 6366–6371 (2012).

424. Dosler, S. & Mataraci, E. In vitro pharmacokinetics of antimicrobial cationic peptides alone and in combination with antibiotics against methicillin resistant Staphylococcus aureus biofilms. *Peptides* **49**, 53–58 (2013).

425. Ghiselli, R. *et al.* Pretreatment With the Protegrin IB-367 Affects Gram-Positive Biofilm and Enhances the Therapeutic Efficacy of Linezolid in Animal Models of Central Venous Catheter Infection. *J. Parenter. Enter. Nutr.* **31**, 463–468 (2007).

426. Cirioni, O. *et al.* The lipopeptides Pal–Lys–Lys–NH2 and Pal–Lys–Lys soaking alone and in combination with intraperitoneal vancomycin prevent vascular graft biofilm in a subcutaneous rat pouch model of staphylococcal infection. *Peptides* **28**, 1299–1303 (2007).

427. Costa, F., Carvalho, I. F., Montelaro, R. C., Gomes, P. & Martins, M. C. L. Covalent immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces. *Acta Biomater.* **7**, 1431–1440 (2011).

428. Onaizi, S. A. & Leong, S. S. J. Tethering antimicrobial peptides: Current status and potential challenges. *Biotechnol. Adv.* **29**, 67–74 (2011).

429. Bagheri, M., Beyermann, M. & Dathe, M. Immobilization reduces the activity of surface-bound cationic antimicrobial peptides with no influence upon the activity spectrum. *Antimicrob. Agents Chemother.* **53**, 1132–41 (2009).

430. Cleophas, R. T. C. *et al.* Convenient Preparation of Bactericidal Hydrogels by Covalent Attachment of Stabilized Antimicrobial Peptides Using Thiol–ene Click Chemistry. *ACS Macro Lett.* **3**, 477–480 (2014).

431. Emanuel, N., Rosenfeld, Y., Cohen, O., Applbaum, Y. H. & Segal, D. A lipid-and-polymer-based novel local drug delivery system—BonyPid™: From physicochemical aspects to therapy of bacterially infected bones. *J. Control. Release* **160**, 353–361 (2012).

432. Twort, F. W. An investigation on the nature of ultramicroscopic viruses. *Lancet* **186**, 1241–1243 (1915).

433. d'Herelle, F. Sur un microbe invisible antagoniste des bacilles dysente´riques (On an invisible microbe antagonistic to dysentery bacilli). *Comptes Rendus l'Acade´mie des Sci.* **165**, 373–375 (1917).

434. D'Herelle, F., Smith, G. H. & Smith, G. H. *The bacteriophage and its behavior / by F. d'Herelle translated by George H. Smith.* (The Williams & Wilkins Company, 1926). doi:https://doi.org/10.5962/bhl.title.7308

435. Dickey, J. & Perrot, V. Adjunct phage treatment enhances the effectiveness of low antibiotic concentration against Staphylococcus aureus biofilms in vitro. *PLoS One* **14**, e0209390 (2019).

436. Donlan, R. M. Preventing biofilms of clinically relevant organisms using bacteriophage. *Trends Microbiol.* **17**, 66–72 (2009).

437. O'Neill, J. *Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations.* (2014).

438. Jorge, P. *et al.* Antimicrobial resistance three ways: healthcare crisis, major concepts and the relevance of biofilms. *FEMS Microbiol. Ecol.* **95**, (2019).

439. Corbin, B. D., McLean, R. J. & Aron, G. M. Bacteriophage T4 multiplication in a glucose-limited Escherichia coli biofilm. *Can. J. Microbiol.* **47**, 680–4 (2001).

440. Hanlon, G. W., Denyer, S. P., Olliff, C. J. & Ibrahim, L. J. Reduction in exopolysaccharide viscosity as an aid to bacteriophage penetration through Pseudomonas aeruginosa biofilms. *Appl. Environ. Microbiol.* **67**, 2746–53 (2001).

441. Adams, M. H. & Park, B. H. An enzyme produced by a phage-host cell system: II. The properties of the polysaccharide depolymerase. *Virology* **2**, 719–736 (1956).

442. Lenski, R. E. & Levin, B. R. Constraints on the Coevolution of Bacteria and Virulent Phage: A Model, Some Experiments, and Predictions for Natural Communities. *Am. Nat.* **125**, 585–602 (1985).

443. Hughes, K. A., Sutherland, I. W. & Jones, M. V. Biofilm susceptibility to bacteriophage attack: the role of phage-borne polysaccharide depolymerase. *Microbiology* **144**, 3039–3047 (1998).

444. Doolittle, M. M., Cooney, J. J. & Caldwell, D. E. Tracing the interaction of bacteriophage with bacterial biofilms using fluorescent and chromogenic probes. *J. Ind. Microbiol.* **16**, 331–341 (1996).

445. Merril, C. R. *et al.* Long-circulating bacteriophage as antibacterial agents. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 3188–92 (1996).

446. Akanda, Z. Z., Taha, M. & Abdelbary, H. Current review-The rise of bacteriophage as a unique therapeutic platform in treating peri-prosthetic joint infections. *J. Orthop. Res.* (2017). doi:https://doi.org/10.1002/jor.23755

447. Furfaro, L. L., Payne, M. S. & Chang, B. J. Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles. *Frontiers in cellular and infection microbiology* **8**, 376 (2018).

448. Berbari, E. F. *et al.* The Mayo Prosthetic Joint Infection Risk Score: Implication for Surgical Site Infection Reporting and Risk Stratification. *Infect. Control Hosp. Epidemiol.* **33**, 774–781 (2012).

449. Dowsey, M. M. & Choong, P. F. M. Early outcomes and complications following joint arthroplasty in obese patients: A review of published reports. *ANZ J. Surg.* **78**, 439–444 (2008).

450. Bongartz, T. *et al.* Incidence and risk factors of prosthetic joint infection after total hip or knee replacement in patients with rheumatoid arthritis. *Arthritis Rheum.* **59**, 1713–20 (2008).

451. Aggarwal, V. K. *et al.* Mitigation and Education. *J. Orthop. Res.* **32**, S16–S25 (2014).

452. Fowler, V. G. *et al.* Effect of an Investigational Vaccine for Preventing Staphylococcus aureus Infections After Cardiothoracic Surgery. *JAMA* **309**, 1368 (2013).

453. McNeely, T. B. *et al.* Mortality among recipients of the Merck V710 Staphylococcus aureus vaccine after postoperative S. aureus infections: An analysis of possible contributing host factors. *Hum. Vaccines Immunother.* (2014). doi:https://doi.org/10.4161/hv.34407

454. Shinefield, H. *et al.* Use of a *Staphylococcus aureus* Conjugate Vaccine in Patients Receiving Hemodialysis. *N. Engl. J. Med.* **346**, 491–496 (2002).

455. Nishitani, K. *et al.* A Diagnostic Serum Antibody Test for Patients With Staphylococcus aureus Osteomyelitis. *Clin. Orthop. Relat. Res.* **473**, 2735–49 (2015).

456. den Reijer, P. M. *et al.* Characterization of the humoral immune response during Staphylococcus aureus bacteremia and global gene expression by Staphylococcus aureus in human blood. *PLoS One* **8**, e53391 (2013).

457. Royan, S. *et al.* Identification of the secreted macromolecular immunogens of *Staphylococcus aureus* by analysis of serum. *FEMS Immunol. Med. Microbiol.* **29**, 315–321 (2000).

458. Dryla, A. *et al.* Comparison of antibody repertoires against Staphylococcus aureus in healthy individuals and in acutely infected patients. *Clin. Diagn. Lab. Immunol.* **12**, 387–98 (2005).

459. Verkaik, N. J. *et al.* Anti-Staphylococcal Humoral Immune Response in Persistent Nasal Carriers and Noncarriers of *Staphylococcus aureus*. *J. Infect. Dis.* **199**, 625–632 (2009).

460. Wheat, J. Diagnostic strategies in osteomyelitis. *Am. J. Med.* **78**, 218–224 (1985).

461. Gedbjerg, N. *et al.* Anti-glucosaminidase IgG in sera as a biomarker of host immunity against Staphylococcus aureus in orthopaedic surgery patients. *J. Bone Joint Surg. Am.* **95**, e171 (2013).

462. Holtfreter, S., Kolata, J. & Bröker, B. M. Towards the immune proteome of Staphylococcus aureus – The anti-S. aureus antibody response. *Int. J. Med. Microbiol.* **300**, 176–192 (2010).

463. Garzoni, C. & Kelley, W. L. Staphylococcus aureus: new evidence for intracellular persistence. *Trends Microbiol.* **17**, 59–65 (2009).

464. Schnaith, A. *et al.* Staphylococcus aureus subvert autophagy for induction of caspase-independent host cell death. *J. Biol. Chem.* **282**, 2695–706 (2007).

465. Kubica, M. *et al.* A Potential New Pathway for Staphylococcus aureus Dissemination: The Silent Survival of S. aureus Phagocytosed by Human Monocyte-Derived Macrophages. *PLoS One* **1**, 1–16 (2008).

466. Tuchscherr, L. *et al.* Staphylococcus aureus phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Mol. Med.* **3**, 129–41 (2011).

467. Hamza, T. & Li, B. Differential responses of osteoblasts and macrophages upon Staphylococcus aureus infection. *BMC Microbiol.* **14**, 207 (2014).

468. Campoccia, D. *et al.* Orthopedic implant infections: Incompetence of Staphylococcus epidermidis, Staphylococcus lugdunensis, and Enterococcus faecalis to invade osteoblasts. *J. Biomed. Mater. Res. – Part A* (2016). doi:https://doi.org/10.1002/jbm.a.35564

469. Vesga, O. *et al.* Staphylococcus aureus small colony variants are induced by the endothelial cell intracellular milieu. *J. Infect. Dis.* **173**, 739–42 (1996).

470. von Eiff, C. *et al.* Intracellular Persistence of Staphylococcus aureus Small-Colony Variants within Keratinocytes: A Cause for Antibiotic Treatment Failure in a Patient with Darier's Disease. *Clin. Infect. Dis.* **32**, 1643–1647 (2001).

471. Clement, S. *et al.* Evidence of an Intracellular Reservoir in the Nasal Mucosa of Patients with Recurrent *Staphylococcus aureus* Rhinosinusitis. *J. Infect. Dis.* **192**, 1023–1028 (2005).

472. Lehar, S. M. *et al.* Novel antibody–antibiotic conjugate eliminates intracellular S. aureus. *Nature* **527**, 323–328 (2015).

473. Zahid, M. & Robbins, P. Cell-Type Specific Penetrating Peptides: Therapeutic Promises and Challenges. *Molecules* **20**, 13055–13070 (2015).
474. Donovan, D. Fusion of peptidoglycan hydrolase enzymes to a protein tranduction domain allow eradication of broth extracellular and intracellular Gram positive pathogens. 1–7 (2013).
475. Donovan, D. M. *et al.* Peptidoglycan hydrolase fusions maintain their parental specificities. *Appl. Environ. Microbiol.* **72**, 2988–96 (2006).
476. Morris, J. *et al.* Evaluation of Bacteriophage Anti-Biofilm Activity for Potential Control of Orthopedic Implant-Related Infections Caused by Staphylococcus Aureus. *Surg. Infect. (Larchmt).* **20**, sur.2018.135 (2018).
477. Koo, H. & Yamada, K. M. Dynamic cell-matrix interactions modulate microbial biofilm and tissue 3D microenvironments. *Curr. Opin. Cell Biol.* **42**, 102–112 (2016).
478. WHO. Surveillance of antimicrobial resistance for local and global action. (2014). Available at: http://www.who.int/drugresistance/events/SwedenMeeting/en/. (Accessed: 1st December 2014)
479. Li, B. & Webster, T. J. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *J. Orthop. Res.* **36**, 22–32 (2018).
480. Davies, S. Antimicrobial resistance poses 'catastrophic threat', says Chief Medical Officer – GOV.UK. *Department of Health and Social Care* (2013). Available at: https://www.gov.uk/government/news/antimicrobial-resistance-poses-catastrophic-threat-says-chief-medical-officer%2D%2D2. (Accessed: 11th March 2019)

Chapter 9
Incidence, Complications and Novel Treatment Strategies: Massive Bone Tumour Surgery

Aadil Mumith and Liza Osagie-Clouard

Abstract The term bone tumour encompasses a broad spectrum of conditions that include both benign and malignant pathologies. Since the 1980s studies have shown that the introduction of an aggressive adjuvant chemotherapy regime improves survival of patients with a malignant bone tumour. Before this time, the standard treatment for a bone malignancy was amputation of the extremity. However, improving patient mortality rates shifted the focus to segmental resection of the tumour with reconstruction using limb salvage techniques. Allograft reconstruction and rotationplasty are employed in limb reconstruction; however, the gold standard for successful functional rehabilitation is use of an endoprosthesis. The risk of postoperative infection of an endoprosthesis is considerably higher when compared with primary total hip and knee replacements. This chapter describes these three reconstructive limb salvage techniques and, while the optimal management of infection remains controversial, presents the consensus statements pertaining to the prevention of musculoskeletal infection in orthopaedic oncology surgery.

Keywords Massive bone tumours · Endoprostheses · Rotationplasty · Allograft · Infection · Treatment · Silver · Novel strategies

9.1 Overview

The term bone tumour encompasses a broad spectrum of conditions that include both benign and malignant pathologies. These tumours affect patients of all ages (Table 9.1) and can be classified based on the dominant tissue within the lesion (Table 9.2), enabling clinicians to target tumour treatment appropriately.

A. Mumith
Sunnybrook Holland Orthopaedic and Arthritic Centre, Toronto, ON, Canada

College of Medicine, University of Central Florida, Orlando, FL, USA

L. Osagie-Clouard (✉)
South West London Elective Orthopaedic Center, London, UK
e-mail: liza.osagie@nhs.net

Table 9.1 Peak age for bone lesions

Age (years)	Benign	Malignant
<20	Fibrous cortical defect, nonossifying fibroma, simple bone cyst, chondroblastoma, Langerhans cell histiocytosis, osteoblastoma, osteofibrous dyplasia, chondromyxoid fibroma, fibrous dysplasia, enchondroma	Leukaemia, Ewing's sarcoma, osteosarcomas, metastatic disease, neuroblastoma, retinoblastoma, rhabdomyosarcoma, Hodgkin's lymphoma
20–40	Enchondroma, giant cell tumour, osteoblastoma, osteoid osteoma, chondromyxoid fibroma, fibrous dysplasia	Osteosarcoma, adamantinoma
>40	Fibrous dysplasia, Paget's disease, non-Hodgkin lymphoma, chondrosarcoma, malignant fibrous histiocytoma, osteosarcomas (secondary to Paget's and radiation)	Metastatic disease (most common), myeloma

Table 9.2 Classification of bone tumours. Revised World Health Organization (WHO) classification

Predominant tissue	Benign	Malignant
Bone forming	Osteoma	Osteosarcoma:
	Osteoid osteoma	Central
	Osteoblastoma	Peripheral
		Parosteal
Cartilage forming	Chondroma	Chondrosarcoma:
	Osteochondroma	Central
	Chondroblastoma	Peripheral
	Chondromyxoid fibroma	Juxtacortical
		Clear-cell
		Mesenchymal
Fibrous tissue	Fibroma	Fibrosarcoma
	Fibromatosis	
Mixed	Chondromyxoid fibroma	
Giant cell tumours	Benign osteoclastoma	Malignant osteoclastoma
Marrow tumours		Ewing's tumour
		Myeloma
Vascular tissue	Haemangioma	Angiosarcoma
	Haemangiopericytoma	Malignant haemangiopericytoma
	Haemangioendothelioma	
Other connective tissue	Fibroma	Fibrosarcoma
	Fibrous histiocytoma	Malignant fibrous histiocytoma
	Lipoma	Liposarcoma
Other tumours	Neurofibroma	Adamantinoma
	Neurilemmoma	Chordoma

Osteosarcomas are the most common bone sarcoma in children and adolescents and represent fewer than 1% of all cancers overall with an incidence of 5 per 1,000,000 in children aged 19 and younger in the USA [1]. Although osteosarcomas affect all ages, there is a clear bimodal distribution with peaks in the pubertal/adolescent patient age group as well as those in their seventh decade. Not only does the tumour location indicate the likelihood of the development of metastases but complete surgical removal is important in order to minimise the risk of further neoplasia [2]. Sarcomas are an aggressive group of tumours, and it has been noted that those who underwent appropriate treatment still did not survive due to metastases. Twenty percent of patients present with signs of metastases; however, the majority of patients with sarcoma have micro-metastases, which can cause disease relapse. Presently, there is an overall 68% survivorship at 5 years for all bone and soft tissue sarcomas [3].

Several studies in the 1980s have shown that the introduction of an aggressive adjuvant chemotherapy regime improves survival of patients with osteosarcoma [4, 5]. Before this time, the standard treatment of osteosarcoma was amputation of the extremity. However improving patient mortality rates shifted the focus to limb salvage involving segmental resection of the tumour with reconstruction using endoprostheses. Custom endoprostheses require time for manufacture that may delay chemotherapy and worsen prognosis, though Rosen et al. [6] demonstrated that the use of neoadjuvant chemotherapy, where patients were treated preoperatively during the time where the custom endoprosthesis was being fabricated, was beneficial. The success of neoadjuvant chemotherapy was reflected in a number of other subsequent studies, which confirmed that this practice was safe, prepared the limb for surgery and further improved mortality rates. As a result neoadjuvant chemotherapy with limb salvage has become the standard treatment of osteosarcomas, and approximately 80% of patients with these tumours are now being treated in this fashion [7].

9.2 Epidemiology

Sarcomas are malignant tumours of connective tissues with bone sarcomas being tumours of the skeleton and soft tissue sarcomas arising from mesenchymal tissue such as muscle, fat and blood vessels to name a few [8]. They represent 1% of all adult cancers, 8% of adolescent cancers and 10% of cancers in children. Despite its rarity, these sarcomas contribute to a large number of years of life lost given the relatively young demographic diagnosed with it [9]. According to the Surveillance, Epidemiology, and End Results (SEER) program, there were 3300 new cases, accompanied by 1490 deaths [3] in the USA during 2016 alone. This type of cancer is most frequently diagnosed in those aged less than 20 years with 27% of new diagnoses belonging to that age group.

9.3 Surgical Treatment Strategies

9.3.1 Allograft Reconstruction

Massive allografts can be used to reconstruct osseo-articular defects left after bone tumour resection. A common location for a primary bone tumour is around the knee, and therefore resection of the tumour would involve loss of major tendon and ligamentous structures as well as some, if not, the whole joint. Allograft reconstruction allows for this defect to be filled with part of a joint and bone from a donor. There are several advantages with the use of biological reconstruction with improved tendon-to-tendon healing as the allograft has preserved soft tissue attachments where the host tendons can be attached to. Allografts avoid the need for massive segmental stemmed implants that may have to cross growth plates and therefore affect limb development. The successful incorporation of an allograft with the host skeleton also increases the bone stock at the site of the initial resection, which can later be used in further reconstructions [10].

There are however disadvantages with the use of allografts. There is a significant shortage in the supply of appropriate massive allografts available for reconstructions in young patients which is an obvious problem given the incidence of bone tumours in this cohort. These allografts cannot be lengthened, and therefore for skeletally immature patients, they are left with limb length discrepancies and will have to undergo further procedures. There is also a high rate of mechanical failure and fracture with autografts coupled with high rates of infection, which is potentially devastating. Grafts may not incorporate with the host bone and therefore fail to produce significant structural integrity [10]. As stabilising ligamentous structures are also removed in certain resections, instability has been observed in 72% of patients with proximal tibial allografts together with joint collapse from cartilage necrosis [11].

In a large series of 945 patients where cadaveric allografts were used for extremity bone and soft tissue tumours, an infection rate of 12.8% was reported. These were highest for patients with soft tissue tumours, radiated sites, Musculoskeletal Tumour Society Stage IIB tumours or those involving an allograft arthrodesis [12]. Infection is the most severe complication leading to failure of graft and usually subsequent amputation [13]. The use of chemotherapy is thought to also increase risk of infection as well as the use of allografts in regions with poor soft tissue coverage that have been exposed to radiotherapy [13], typically those involving tibial reconstructions are twice as likely to become infected when compared with distal femur [14, 15].

9.3.2 Rotationplasty

Initially described by Borggreve et al. [16] for patients left with limb deformities as a sequelae of tuberculosis, its popularity has diminished with the advent of improving implant designs. It still has a role as an option for failed limb salvage

procedures. It involves the use of the lower leg below the knee as a surrogate for the distal femur with the foot rotated around to face the opposite direction, and hence the ankle is used to replace the knee joint. This allows for less energy expenditure during ambulation compared with above knee amputations [17]. This procedure avoids phantom limb pain, the need for further limb lengthening procedures, revision surgery for failed prostheses and loosening [10].

Complications of rotationplasty include delayed healing and infection. Vascular compromise has been reported being as high as 12% which would lead to amputation. Rotationplasty is an option which exists for those cases where limb salvage is not possible or has failed with amputation being the only other remaining option [10]. Small case series are available in the literature describing infection rates; however with the limited heterogenous cohort sizes, it is difficult to delineate risk factors for this [18–20].

9.3.3   Endoprosthesis

Austin-Moore created the first metallic endoprosthesis using an alloy known as Vitallium [21] to reconstruct a proximal femur following resection of a giant cell tumour. Radiographs at 1 year showed extracortical bone formation around the shaft of the implant. Following this success, the development of endoprostheses had been started using Vitallium as well as other materials [22–28]. However success was still limited, and at this time, amputation remained the gold standard in managing bone tumours.

Due to technological advances in various medical fields and improvements in the life expectancy of musculoskeletal tumour patients, advances in the development and manufacture of orthopaedic tumour implants grew rapidly in the 1970s. The advent of the modern tumour endoprosthesis had started with clinicians using preoperative chemotherapy in conjunction with endoprosthetic reconstruction allowing improved survival rates together with limb salvage [29–31]. Neoadjuvant and adjuvant chemotherapy is currently the most common form of chemotherapy used in musculoskeletal oncology.

Developments in material science in producing titanium (Ti) alloys, most notably Ti_6Al_4V, paved the way for an improvement in endoprosthesis performance in resisting corrosion [32] and silver coating in reducing rates of infection [33]. This has led endoprostheses to be the main choice in limb reconstruction for patients provided the tumour had been resected with satisfactory margins. This allows for successful functional rehabilitation with local recurrence rates being similar to amputation [34] making endoprosthetic reconstruction and limb salvage the gold standard in the management of primary bone tumours.

The use of endoprostheses was popularised in the 1990s. These endoprostheses were either cemented or uncemented. John Charnley popularised the use of bone cement in the 1970s [35], and his principles were applied to the fixation of endoprostheses, which was comprised of an intramedullary stem continuous with the

implant being cemented into the canal of the remaining bone. It became apparent that these cemented implants were becoming loose at the cement-implant interface leading to osteolysis of the surrounding bone. Cortical bone loss is seen initially at the point of direct contact between the bone and the shoulder of the implant. This is followed by worsening osteolysis thought to be induced by wear debris from the polymethylmethacrylate cement, which is commonly used for the initial fixation of the implant [36]. Loosening then led to implant failure although that it was not the sole cause. Wirganowicz et al. [37] first described the causes of endoprosthetic failure and categorised them into mechanical and non-mechanical causes. Henderson et al. further developed upon this to classify endoprosthetic failure into five different modalities: soft tissue failure (type I), aseptic loosening (type II), structural failure (type III), infection (type IV) and tumour progression (type V) [38].

9.4 Endoprosthetic Periprosthetic Infection

The risk of postoperative infection of endoprostheses is considerably higher than those of primary total hip and knee replacements [39, 40]. This high rate of infection is attributed to multiple factors which include those involving the patient, the technical aspects of the procedure and the postoperative course. Patient factors are especially important in orthopaedic oncological reconstructions as the majority of these individuals are immunocompromised secondary to chemotherapy regimens. The International Consensus Meeting on Musculoskeletal Infection has made a number of recommendations. Table 9.3 summarises these consensus statements related to the prevention of infection in orthopaedic oncology surgery [41]. Diagnosing infection is challenging and follows similar algorithms used for standard joint replacements. Following assessment with radiographs and other imaging modalities in conjunction with serum markers, the gold standard is periprosthetic tissue cultures as well as joint fluid aspiration [42–45].

A variety of bacteria can cause periprosthetic joint infections (PJI), the most common being gram-positive bacteria accounting for 60–80% of PJI [46]. In polymicrobial infections causing PJI, gram-negative bacteria have been reported to play a role. Poorer outcomes are seen when PJI involves multidrug-resistant bacteria [46]. Primary arthroplasty has a known infection rate of 1–2% although in comparison, the infection rate associated with endoprosthesis use is 8–15% with some studies suggesting as high as 40% [39, 47]. This increased rate of infection is due to increased host risk factors as those receiving such implants may be immunocompromised, a result of chemotherapy regimens being used to treat the primary sarcoma. Increased blood loss, operative time, soft tissue trauma and dead space are further reasons why there is an increased rate of PJI [39, 41, 48, 49]. In a cohort of 4495 patients, Nucci et al. [50] found an overall PJI rate of 14.1%, 47.6% of these were associated with distal femoral replacements with 30% in proximal tibial replacements. It is thought that those endoprostheses around the knee have increased risk of infection due to a compromised soft tissue envelope. Ninety percent of cases in

Table 9.3 Consensus statements pertaining to the prevention of musculoskeletal infection in orthopaedic oncology surgery

Question	Consensus statement	Level of evidence	Consensus note
Is there a correlation between operative time and the risk of subsequent SSI/PJI in patients undergoing tumour resection and endoprosthetic reconstruction? If so, should postoperative antibiotics be prolonged in these patients?	Based largely on the arthroplasty literature, there is considerable evidence that prolonged operative time is associated with an increased risk for postoperative infection.	Moderate	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
	However, there is insufficient evidence to suggest that a prolonged postoperative antibiotic regimen can mitigate this risk. Therefore, there is no evidence to support prolonged postoperative antibiotics in orthopaedic oncology patients undergoing surgeries of prolonged duration. If the duration of the procedure exceeds two half-lives of the prophylactic antimicrobial, intraoperative redosing is needed to ensure adequate serum and tissue concentrations of the antimicrobial		
Should factors like preoperative radiation, soft tissue versus bone resection, presence of metal versus structural allograft and other factors influence the dose and duration of antibiotic prophylaxis?	Unknown. Evidence and guidelines directing the prescription of prophylactic antibiotic regimens in musculoskeletal tumour surgery are lacking. Although long-term antibiotic prophylaxis may decrease the risk of deep infection, there is not sufficient evidence to recommend the use of anything other than routine antibiotic prophylaxis for patients undergoing major reconstruction	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Should patients with an oncologic endoprosthesis in place receive antibiotic prophylaxis during dental procedures?	Not routinely. Evidence-based guidelines by dentists and orthopaedic surgeons state that antibiotic prophylaxis is rarely appropriate for patients with prosthetic joints	Consensus	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Should prophylactic antibiotics be started in patients with an oncologic endoprosthesis who develop neutropenia secondary to postoperative chemotherapy?	Not routinely. Evidence-based guidelines recommend limiting the routine use of prophylactic antibiotics to high-risk patients with chemotherapy-induced neutropenia	Consensus	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)

Table 9.3 (continued)

Question	Consensus statement	Level of evidence	Consensus note
What type, dose and duration of prophylactic antibiotic(s) should be administered to patients undergoing oncologic endoprosthetic reconstruction who have received or will be receiving chemotherapy and/or radiation?	Antibiotic prophylaxis should be given in accordance with existing guidelines for standard arthroplasty surgery and other orthopaedic surgical procedures with foreign body placement	Consensus	Agree 93% Disagree 0% Abstain 7% (super majority, strong consensus)
Does the type, dose and duration of antibiotic prophylaxis differ for patients undergoing oncologic endoprosthetic reconstruction compared with conventional TJA?	No. There is no recommendation to adjust type, dose or duration of antibiotic prophylaxis in patients undergoing oncologic endoprosthetic reconstruction from that which is routinely administered in conventional TJA	Consensus	Agree 93% Disagree 0% Abstain 7% (super majority, strong consensus)
Do we need to evaluate the gut and skin microbiome of patients after chemotherapy to assess the risk for potential infection after endoprosthetic reconstruction?	Unknown. There is no evidence in the literature to suggest that evaluation of the gut and/or skin microbiome following chemotherapy aids with risk stratification for potential infection in patients undergoing endoprosthetic limb salvage surgery	Consensus	Disagree 0% Abstain 0% (unanimous, strongest consensus)
Should an absolute neutrophil count of >1000/mm^3 be the minimum for patients undergoing limb salvage surgery after receiving chemotherapy?	Yes. An absolute neutrophil count of >1000/mm^3 should be the minimum for patients undergoing limb salvage surgery after receiving chemotherapy	Consensus	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Should the serum WBC count be taken into account prior to endoprosthetic reconstruction in patients who have undergone recent chemotherapy?	The association between chemotherapy and infection following endoprosthetic reconstruction remains controversial. However, in a multifactorial decision-making process, there may be some benefit in accounting for the serum WBC count prior to endoprosthetic reconstruction	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)

Table 9.3   (continued)

Question	Consensus statement	Level of evidence	Consensus note
What should be the time delay between preoperative chemo-/radiation therapy and a surgical tumour resection in order to minimize incidence of SSI/PJI?	Unknown. There is no data that support the best time delay between preoperative chemo-/radiation therapy and a surgical tumour resection to minimise the incidence of SSI/PJI. There are multiple intrinsic factors in each patient that can determine the best time to implant an endoprosthesis after a neoadjuvant treatment. Although no significance was seen between preoperative radiation therapy and surgical timing on wound complications, trends suggest rates are lower if surgery is performed between 3 and 6 weeks following radiation therapy	Consensus	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
What strategies should be implemented to minimise the risk of SSI/PJI in patients who have received chemotherapy or radiation therapy and are undergoing endoprosthetic reconstruction?	We believe patients who have received either chemotherapy or radiation therapy prior to endoprosthetic reconstruction should undergo extensive medical optimisation.	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
	Consideration may also be given to the use of antimicrobial coated implants, extended (>24 h) and augmented postoperative antibiotic prophylaxis consisting of a first-generation cephalosporin and an aminoglycoside and/or vancomycin, as well as use of enhanced soft tissue reconstruction techniques. Surgery should also be expeditious in these patients, minimising dissection of soft tissues with gentle handling		

Table 9.3 (continued)

Question	Consensus statement	Level of evidence	Consensus note
What are the substantial risk factors for SSI/PJI of an oncologic endoprosthesis following resection of a malignant bone tumour?	Patient-related risk factors for SSI/PJI of an oncologic endoprosthesis include increased patient body mass index, overall presence of comorbidities, coexistence of superficial SSI or skin necrosis and lower preoperative haemoglobin or albumin levels. Disease-related risk factors for SSI/PJI of an oncologic endoprosthesis include lesion localisation in proximal tibia, pelvis and lesion extending to pelvis from proximal femur. In addition, procedure-related risk factors for SSI/PJI include preoperative hospitalisation longer than 48 hours, resection of >37% of the proximal aspect of the tibia, resection of three or four heads of the quadriceps muscle in distal femoral lesions compared with one or two heads, increasing surgical time (>2.5 h), use of a cemented oncologic endoprosthesis, need for postoperative admission to the intensive care unit, increased postoperative blood transfusion requirement (‡2 units of allogeneic packed cells), presence of postoperative haematoma and the need for additional surgical procedures after the megaprosthesis implantation	Moderate	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
What metrics should be used to determine the optimal timing of reimplantation for patients with a resected oncologic endoprosthesis?	Prior to reimplantation of an oncologic endoprosthesis after a previous resection, surgeons must ensure that the infection has been eradicated from the surgical bed. This would be determined via a sterile aspirate from the joint cavity following the antibiotic treatment	Moderate	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Is there an increased risk for subsequent SSI/PJI when a drainage tube is used in musculoskeletal tumour surgery?	Surgical drains should be used selectively in patients undergoing musculoskeletal tumour surgery. If used, they should be continuously monitored and removed immediately once output has decreased adequately per clinical judgement.	Limited	Agree 100% Disagree 0% Abstain: 0% (unanimous, strongest consensus)
	There is a potential, yet unproven, link between the use of surgical drains and increased risk of SSI/PJI following orthopaedic procedures involving the use of prostheses		

Table 9.3 (continued)

Question	Consensus statement	Level of evidence	Consensus note
When should a surgical drain be removed to minimise the risk of subsequent SSI/PJI in patients who have received endoprosthetic reconstruction following resection of a musculoskeletal tumour?	Based on the available literature, we recommend drains be removed within 24 h of surgery	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Does the type of fixation (cemented versus uncemented) of an oncologic endoprosthesis influence the incidence of subsequent SSI/PJI?	There is conflicting evidence surrounding this topic. Multiple studies have demonstrated superiority with cemented fixation of an oncologic endoprosthesis, while others have suggested superiority with uncemented fixation. Therefore, the choice of the method of fixation should be made on the basis of all clinical indications other than the influence of fixation on subsequent SSI/PJI	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Does the use of incise drapes with antibacterial agents (iodine) influence the risk for subsequent SSI/PJI in patients undergoing musculoskeletal tumour surgeries?	There is some evidence claiming that antimicrobial impregnated incise drapes result in a reduction in bacterial contamination at the surgical site. However, there is little evidence to demonstrate that it results in a subsequent reduction in the incidence of SSI and/or PJI	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Does the use of soft tissue attachment meshes increase the risk for subsequent PJI in patients undergoing oncologic endoprosthetic reconstruction?	The current literature indicates that there is no increased risk of PJI in this patient population with the use of soft tissue attachment meshes. However, there are few studies directly comparing the use of mesh versus not using mesh in comparable tumours/surgical locations, so additional comprehensive study on the topic is necessary to say with reasonable certainty that there is no connection	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Should an endosprosthesis and/or allograft bone be soaked in antibiotic solution or antiseptic solutions prior to implantation in patients?	Unknown. There is no evidence to suggest that the use of a preimplantation antibiotic or the antiseptic soak of an endoprosthesis or massive allograft would reduce the rate of SSI/PJI	Consensus	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)

Table 9.3 (continued)

Question	Consensus statement	Level of evidence	Consensus note
Should a coated prosthesis (silver/iodine) be used for reconstruction of patients undergoing primary bone tumour resection?	Yes. Silver coating and iodine coating of a prosthesis show good results in prevention of infection after reconstruction following primary tumour resection	Moderate	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
What is the most optimal local antimicrobial delivery strategy during limb salvage: antibiotic cement, silver-coated implant, iodine-coated implant, topical vancomycin powder, injection of antibiotics via drain tubing or other?	Unknown. No direct comparison has been made of different antimicrobial delivery strategies in oncology patients undergoing limb salvage procedures	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
How many I&Ds of an infected oncologic endoprosthesis are reasonable before consideration should be given to resection arthroplasty?	Decision to repeat irrigation and debridement and retention of an infected endoprosthesis (DAIR) should be made based on comorbidities of the host, virulence of the organism, complexity of the reconstruction and status of the soft tissues. We believe that DAIR performed more than two or three times is unlikely to be successful	Consensus	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
How should acute reinfection of an oncologic endoprosthesis be treated?	Acute reinfections in patients with oncologic endoprostheses demand treatment by surgical methods because the long-term administration of antibiotics alone is not sufficient. The most appropriate treatment modality for acute reinfection is DAIR with exchange of components	Consensus	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Is I&D and exchange of modular parts a viable option for treatment of acute PJI involving an oncologic endoprosthesis? If so, what are the indications?	Yes. Irrigation and debridement with retention of prosthesis (DAIR) is a viable option for management of patients with an infected endoprosthesis. The procedure may be offered to patients with superficial early infection (<3 months), short duration of symptoms (<3 weeks), well-fixed implants and a well-characterised organism demonstrating a highly susceptible pathogen	Moderate	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)

Table 9.3   (continued)

Question	Consensus statement	Level of evidence	Consensus note
Does the use of iodine-coated or silver-coated implants make one-stage exchange arthroplasty possible in the management of patients with an infected oncologic endoprosthesis?	Unknown. Current literature has advocated the advantages of surface-modified coating (e.g. silver-coated iodine-supported implants). Recently, there have been several low-quality small-scale studies showing promising results for using surface-modified implants in one-stage exchange arthroplasty to treat an infected oncologic endoprosthesis. However, to date, there remains unsubstantiated evidence, and large-scale high-level evidence studies are necessitated	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Is there a role for single-stage exchange arthroplasty for patients with an infected oncologic endoprosthesis?	In principle, despite the lack of sufficient evidence, single-stage exchange arthroplasty can be performed in patients with an infected oncologic endoprosthesis if the general requirements to perform a single-stage procedure are fulfilled. However, a single-stage revision without removing the anchorage components is not recommended since better infection control can be achieved when prostheses are removed rather than salvaged	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
Should the management of a PJI involving an oncologic endoprosthesis differ from that of conventional joint replacement prostheses?	No. The management of a PJI involving an oncologic endoprosthesis is similar to that of conventional joint replacement prosthesis	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)
What factors may improve the outcome of a two-stage exchange arthroplasty in patients with an infected oncologic endoprosthesis?	There are numerous factors that improve the outcome of two-stage exchange arthroplasty in general and after oncologic reconstruction in particular. These include host-related factors (such as host optimisation by treating anaemia, malnutrition, hyperglycaemia, immunosuppressive state, etc.), organism-related factors (e.g. administration of appropriate systemic and local antibiotics) and surgery-related factors (e.g. aggressive debridement of soft tissue and bone, optimal soft tissue management and prevention of postoperative complications)	Limited	Agree 100% Disagree 0% Abstain 0% (unanimous, strongest consensus)

Table 9.3 (continued)

Question	Consensus statement	Level of evidence	Consensus note
What is the best reconstruction technique for an infected allograft?	The best reconstruction technique for an infected allograft is resection of the infected allograft and reconstruction (preferably, two-stage) with an endoprosthesis	Moderate	Agree 93% Disagree 0% Abstain 7% (super majority, strong consensus)
What is the best surgical treatment for management of a chronically infected oncologic endoprosthesis? Does this change if the patient is receiving or has received recent chemotherapy and/or irradiation?	We recommend a two-stage revision in the management of a chronically infected oncologic endoprosthesis; however, we acknowledge that support for a one-stage exchange is increasing. There is no study to suggest that this recommendation should change if the patient is receiving or has received recent chemotherapy and/or irradiation	Limited	Agree 93% Disagree 0% Abstain 7% (super majority, strong consensus)

I&D irrigation and debridement, *DAIR* debridement, antibiotics and implant retention, *PJI* periprosthetic joint infection [41]

the cohort isolated a pathogen with *Staphylococcus epidermidis* (21%) and *S. aureus* (13.5%) which were the most common.

The optimal management of endoprostheses remains controversial and ranges from debridement, antibiotics and implant retention (DAIR), one- or two-stage revision, excision arthroplasty and finally amputation [39].

9.5 Novel Strategies

9.5.1 A Silver Coating to Combat Infection

Prosthetic joint infections are one of the most common complications in endoprosthetic reconstruction, with an incidence reportedly as high as 30% in primary and 60% in revision reconstructions [51, 52]; currently, two-stage revision with surgical debridement is the mainstay of treatment [53–55]. Developments have increased the option for antimicrobial surfaces which include antibiotic-based coatings, chitosan coatings, antiseptic coatings, photoactive-based coatings and silver coatings of implants to combat infection. Antibiotic coatings have been widely studied and are easy to obtain, but are hampered by limited length of elution and bacterial resistance. Similarly, antiseptic coatings such as chlorhexidine and chloroxylenol have also shown in vivo efficacy, but are hampered by local and systemic toxicity.

Silver particles combine antimicrobial activity with low cell toxicity, as such, studies demonstrate that coating the prosthesis significantly reduced infection rates. Silver may be 'stitched' into the implant surface by anodisation and subsequent dipping in aqueous silver solution or combining the silver layer with a layer of argentum [56, 57]. Whereby, the resultant cathodic reaction produces a proton depleting region around the prosthesis and has been seen to alter the transmembrane proton gradient, reduce intracellular ATP synthesis and subsequently induce bacterial apoptosis [58–60]. Other methods such as a multilayer silver coating or combined porous argentums consist of two layers: a deep basic layer of silver (1 lm-thick) and a hard-top layer of TiAg20 N (0.1 lm-thick) have also demonstrated positive outcomes in reducing infection persistence, but also in prevalence.

Studies investigating silver-coated megaprostheses have mainly used them in cases of previous periprosthetic infection or other revision surgeries and not as the primary implant [61, 62, 63, 64]. Glehr et al. [61] reported an infection rate of 12.5% in 32 patients treated with a silver-coated MUTARS (modular universal tumour and revision system) tumour endoprosthesis. Wafa et al. [64] compared infection rates between an uncoated tumour prosthesis (Stanmore Implants) and a silver-coated implant (Agluna, Stanmore Implants). A significantly lower reinfection rate was noted after a two-stage revision with silver-coated implants (15%) in comparison with uncoated implants (42.9%). Studies comparing primary coated endoprosthetics have also demonstrated a significant difference, with an 8.9% infection rate found in silver-coated primary tibial implants compared to up to 16.7% in titanium only prosthetics [65]. Additionally, infections that did occur in the silver-coated group required less invasive treatment and fewer operative interventions, as such the use of a silver coating becoming more widespread.

Several side effects have been reported in earlier studies, including argyria, kidney and liver damage, leucopoenia and toxicity in neural tissues [19, 24, 25]. These effects have been described at blood concentrations exceeding 300 ppb, though a therapeutic bactericidal effect is already seen at very low concentrations (starting from 35 ppb). As such the use of a silver coating on massive endoprosthetics is becoming increasingly widespread.

9.5.2 Additive Manufacturing

Increasing innovation in additive manufacturing (AM), also known as three-dimensional (3D) printing and rapid prototyping, is bringing about a paradigm shift in translational medico-surgical research. This novel technology allows for the manufacturing of objects with complex geometries. A completely porous collar can be produced using AM techniques. An open porous structure enables the bone in growth into the collar forming a stronger bond when compared with just surface on growth. In theory the bone can grow directly from the cortical bone at the transection site into the porous structure. AM allows the complete control over the specifications of the porous metal. The pore size and shape as well the strut size can be

controlled highly accurately. As a result the biomechanical properties of the implant can be controlled to more closely mimic that of the bone, without the cost and time implications of fully custom implants. Direct bone contact is necessary to allow for effective stress transfer [66]. 3D-printed fully porous implants have been shown to reduce peri-implant osteolysis secondary amount of bone loss secondary to stress shielding by 75% compared to a conventional fully solid implant, demonstrating the merit and potential of modifying material architecture to combat stress-induced bone resorption [67]. Further studies [67, 68] have supported these promising results, comparing AM to standard hemipelvis prosthetics, and at a mean follow-up, comparative survivorship and improved clinical outcome scores were noted with AM implants, concluding that 3D-printed pelvic prostheses facilitated precision matching and aided osseointegration between implants and the host bone. In addition, bioactive coatings as an adjunct to additive manufacturing are also improving endoprosthesis survival. Plasma spraying is a technique that will coat the outer surface of a 3D-printed scaffold, depositing the coating electrochemically to allow even coverage.

9.6 Conclusion

Currently, advances in the surgical management and techniques for sarcomas continue to be driven by advances in imaging and implant design. The periprosthetic infection or loosening of megaprostheses can be catastrophic, leading to significant morbidity, while fracture and non-union of large allografts, vascularised or otherwise, can result in multiple surgical interventions and associated socioeconomic costs. Innovations in additive manufacturing and the implant surface – be it to enhance osseointegration or sintered silver to inhibit bacterial activity – are improving surgical outcomes and restoring quality of life to sarcoma patients.

References

1. Anderson, M.E., Update on survival in osteosarcoma. Orthopedic Clinics of North America, 2016. 47(1): p. 283–292.
2. Meyers, P.A., et al., Osteogenic sarcoma with clinically detectable metastasis at initial presentation. Journal of Clinical Oncology, 1993. 11(3): p. 449–453.
3. Howlader, N., et al. *SEER Cancer Statistics Review*. 1975–2013 based on November 2015 SEER data submission]; Available from: http://seer.cancer.gov/csr/1975_2013/.
4. Eilber, F., et al., Adjuvant chemotherapy for osteosarcoma: a randomized prospective trial. J Clin Oncol, 1987. 5(1): p. 21–6.
5. Link, M.P., et al., The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. N Engl J Med, 1986. 314(25): p. 1600–6.
6. Rosen, G., et al., Primary osteogenic sarcoma: the rationale for preoperative chemotherapy and delayed surgery. Cancer, 1979. 43(6): p. 2163–77.

7. Allison, D.C., et al., A meta-analysis of osteosarcoma outcomes in the modern medical era. Sarcoma, 2012. 2012: p. 704872.

8. Fletcher, C.D., K.K. Unni, and F. Mertens, *Pathology and genetics of tumours of soft tissue and bone*. Vol. 4. 2002: Iarc.

9. Amankwah, E.K., A.P. Conley, and D.R. Reed, Epidemiology and therapies for metastatic sarcoma. Clin Epidemiol, 2013. 5: p. 147-162.

10. Levin, A.S., A. Arkader, and C.D. Morris, Reconstruction Following Tumor Resections in Skeletally Immature Patients. Journal of the American Academy of Orthopaedic Surgeons, 2017. 25(3): p. 204–213.

11. Muscolo, D.L., et al., Proximal Tibia Osteoarticular Allografts in Tumor Limb Salvage Surgery. Clinical Orthopaedics and Related Research, 2010. 468(5): p. 1396–1404.

12. Mankin, H.J., F.J. Hornicek, and K.A. Raskin, *Infection in massive bone allografts.* Clinical Orthopaedics and Related Research®, 2005. **432**: p. 210–216.

13. Matejovsky, Z. and I. Kofranek, Massive allografts in tumour surgery. International orthopaedics, 2006. 30(6): p. 478–483.

14. Rabitsch, K., et al., Intercalary reconstructions with vascularised fibula and allograft after tumour resection in the lower limb. Sarcoma, 2013. 2013.

15. Bus, M., et al., Is there still a role for osteoarticular allograft reconstruction in musculoskeletal tumour surgery? A long-term follow-up study of 38 patients and systematic review of the literature. The bone & joint journal, 2017. 99(4): p. 522–530.

16. Borggreve, J., Kniegelenksersatz durch das in der Beinlängsachse um 180 gedrehte Fußgelenk. Arch Orthop Unfallchir, 1930. 28: p. 175–178.

17. van der Windt, D.A., et al., Energy expenditure during walking in subjects with tibial rotationplasty, above-knee amputation, or hip disarticulation. Archives of physical medicine and rehabilitation, 1992. 73(12): p. 1174–1180.

18. Sawamura, C., et al., Indications for and surgical complications of rotationplasty. Journal of Orthopaedic Science, 2012. 17(6): p. 775–781.

19. Agarwal, M., et al., *Rotationplasty for bone tumors: is there still a role?* Clinical Orthopaedics and Related Research (1976–2007), 2007. **459**: p. 76–81.

20. Kinoshita, H., et al., Effectiveness of Salvage Knee Rotationplasty on Sarcoma Around the Knee in Adolescents and Young Adults. Anticancer Research, 2021. 41(2): p. 1041–1046.

21. Moore, A.T., Metal hip joint; a new self-locking vitallium prosthesis. Southern medical journal, 1952. 45(11): p. 1015.

22. Brav, E.A., F.J. Mc, and J.A. Miller, The replacement of shaft defects of long bones by metallic prostheses. Am J Surg, 1958. 95(5): p. 752–60.

23. Horwitz, T., Use of a shaft prosthesis in the treatment of surgically resistant nonunion of the humerus. Bull Hosp Joint Dis, 1955. 16(1): p. 37–44.

24. Loomis, L.K., *Internal prosthesis for upper portion of femur; a case report.* J Bone Joint Surg Am, 1950. **32 a**(4): p. 944–6.

25. Macausland, W.R., Replacement of the lower end of the humerus with a prosthesis; a report of four cases. West J Surg Obstet Gynecol, 1954. 62(11): p. 557–66.

26. Moore, A.T., *The self-locking metal hip prosthesis.* J Bone Joint Surg Am, 1957. **39-a**(4): p. 811–27.

27. Seddon, H.J. and J.T. Scales, A polythene substitute for the upper two-thirds of the shaft of the femur. Lancet, 1949. 2(6583): p. 795.

28. Venable, C.S., An elbow and an elbow prosthesis; case of complete loss of the lower third of the humerus. Am J Surg, 1952. 83(3): p. 271–5.

29. Huvos, A.G., G. Rosen, and R.C. Marcove, Primary osteogenic sarcoma: pathologic aspects in 20 patients after treatment with chemotherapy en bloc resection, and prosthetic bone replacement. Arch Pathol Lab Med, 1977. 101(1): p. 14–8.

30. Rosen, G., et al., Chemotherapy, en bloc resection, and prosthetic bone replacement in the treatment of osteogenic sarcoma. Cancer, 1976. 37(1): p. 1–11.

31. Sinks, L.F. and E.R. Mindell, Chemotherapy of osteosarcoma. Clin Orthop Relat Res, 1975(111): p. 101–4.
32. Golish, S.R. and W.M. Mihalko, Principles of biomechanics and biomaterials in orthopaedic surgery. J Bone Joint Surg Am, 2011. 93(2): p. 207–12.
33. Ghani, Y., et al., Development of a hydroxyapatite coating containing silver for the prevention of peri-prosthetic infection. J Orthop Res, 2012. 30(3): p. 356–63.
34. Grimer, R.J., A.M. Taminiau, and S.R. Cannon, Surgical outcomes in osteosarcoma. J Bone Joint Surg Br, 2002. 84(3): p. 395–400.
35. Charnley, J., Total hip replacement by low-friction arthroplasty. Clin Orthop Relat Res, 1970. 72: p. 7–21.
36. Ward, W.G., et al., Loosening of massive proximal femoral cemented endoprostheses. Radiographic evidence of loosening mechanism. J Arthroplasty, 1997. 12(7): p. 741–50.
37. Wirganowicz, P.Z., et al., Etiology and results of tumor endoprosthesis revision surgery in 64 patients. Clin Orthop Relat Res, 1999(358): p. 64–74.
38. Henderson, E.R., et al., Failure mode classification for tumor endoprostheses: retrospective review of five institutions and a literature review. J Bone Joint Surg Am, 2011. 93(5): p. 418–29.
39. Sigmund, I.K., et al., Efficacy of different revision procedures for infected megaprostheses in musculoskeletal tumour surgery of the lower limb. PLoS One, 2018. 13(7): p. e0200304.
40. Pilge, H., et al., *Incidence and outcome after infection of megaprostheses.* Hip International, 2012. **22**(8_suppl): p. 83–90.
41. Strony, J., et al., Musculoskeletal infection in orthopaedic oncology: assessment of the 2018 International Consensus Meeting on Musculoskeletal Infection. JBJS, 2019. 101(20): p. e107.
42. Ercolano, L.B., et al., *Treatment solutions are unclear for perimegaprosthetic infections.* Clinical Orthopaedics and Related Research®, 2013. **471**(10): p. 3204–3213.
43. Sambri, A., et al., Sonication improves the diagnosis of Megaprosthetic infections. Orthopedics, 2019. 42(1): p. 28–32.
44. Jeys, L., et al., Periprosthetic infection in patients treated for an orthopaedic oncological condition. JBJS, 2005. 87(4): p. 842–849.
45. Peel, T., et al., Infective complications following tumour endoprosthesis surgery for bone and soft tissue tumours. European Journal of Surgical Oncology (EJSO), 2014. 40(9): p. 1087–1094.
46. Papadopoulos, A., et al., Multidrug-resistant and extensively drug-resistant Gram-negative prosthetic joint infections: Role of surgery and impact of colistin administration. International journal of antimicrobial agents, 2019. 53(3): p. 294–301.
47. Morii, T., et al., Deep infection in tumor endoprosthesis around the knee: a multi-institutional study by the Japanese musculoskeletal oncology group. BMC musculoskeletal disorders, 2013. 14(1): p. 1–9.
48. Kapoor, S.K. and R. Thiyam, Management of infection following reconstruction in bone tumors. Journal of clinical orthopaedics and trauma, 2015. 6(4): p. 244–251.
49. Zajonz, D., et al., Periprosthetic joint infections in modular endoprostheses of the lower extremities: a retrospective observational study in 101 patients. Patient safety in surgery, 2016. 10(1): p. 1–9.
50. Nucci, N., et al., Management of infected extremity endoprostheses: a systematic review. European Journal of Orthopaedic Surgery & Traumatology, 2020. 30: p. 1139–1149.
51. Henderson ER, O'Connor MI, Ruggieri P, Windhager R, Funovics PT, Gibbons CL, Guo W, Hornicek FJ, Temple HT, Letson GD (2014) Classification of failure of limb salvage after reconstructive surgery for bone tumours: a modifed system including biological and expandable reconstructions. Bone Joint J 96-B(11):1436–1440
52. Flint MN, Grifn AM, Bell RS, Wunder JS, Ferguson PC (2007) Two-stage revision of infected uncemented lower extremity tumor endoprostheses. J Arthroplast 22(6):859–865
53. Ercolano LB, Christensen T, McGough R, Weiss K (2013) Treatment solutions are unclear for perimegaprosthetic infections. Clin Orthop Relat Res 471(10):3204–3213

54. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR (2013) Infectious Diseases Society of A. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis Of Publ Infect Dis Soc Am 56(1):e1–e25 12.
55. Parvizi J, Gehrke T (2014) International consensus group on periprosthetic joint I. Definition of periprosthetic joint infection. J Arthroplast 29(7):1331
56. Scoccianti, G, Frenos, F, Beltrami, G. Levels of silver ions in body fluids and clinical results in silver-coated megaprostheses after tumour, trauma or failed arthroplasty. Injury 2016; 47(Suppl. 4): S11–S16
57. Parry MC, Laitinen MK, Albergo JI, et al. Silver-coated (Agluna®) tumour prostheses can be a protective factor against infection in high risk failure patients. European Journal of Surgical Oncology: the Journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 2019 Apr;45(4):704–710.
58. Yamanaka, M, Hara, K, Kudo, J. Bactericidal actions of a silver ion solution on Escherichia coli, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl Environ Microbiol 2005; 71: 7589–7593.
59. Jung, WK, Koo, HC, Kim, KW. Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Appl Environ Microbiol 2008; 74: 2171–2178.
60. Morones, JR, Elechiguerra, JL, Camacho, A. The bactericidal effect of silver nanoparticles. Nanotechnology 2005; 16: 2346–2353
61. Glehr M, Leithner A, Friesenbichler J et al (2013) Argyria fol-lowing the use of silver-coated megaprostheses: no association between the development of local argyria and elevated silver-levels. Bone Joint J 95(7):988–99229.
62. Scoccianti G, Frenos F, Beltrami G, Campanacci DA, Capanna R(2016) Levels of silver ions in body fluids and clinical results insilver-coated megaprostheses after tumour, trauma or failedarthroplasty. Injury 47(Supplement 4):S11–S1630.
63. Donati F, Di Giacomo G, D'Adamio S et al (2016) Silver-coatedhip megaprosthesis in oncological limb savage [sic] surgery. Biomed Res Int 2016:6,31.
64. Wafa H, Grimer RJ, Reddy K et al (2015) Retrospective evalu-ation of the incidence of early periprosthetic infection with silver-treated endoprostheses in high-risk patients: case-control study. Bone Joint J 97(2):252–257
65. Hardes J, Henrichs MP, Hauschild G, Nottrott M, Guder W, Streitbuerger A. Silver-Coated Megaprosthesis of the Proximal Tibia in Patients With Sarcoma. J Arthroplasty. 2017 Jul;32(7):2208–2213
66. Vee San Cheong, Paul Fromme, Melanie J. Coathup, Aadil Mumith, Gordon W. Blunn. Partial Bone Formation in Additive Manufactured Porous Implants Reduces Predicted Stress and Danger of Fatigue Failure. Annals of Biomedical Engineering. 2020, 48, 502–514
67. Arabnejad S, Johnston B, Tanzer M, Pasini D. Fully porous 3D printed titanium femoral stem to reduce stress-shielding following total hip arthroplasty. J Orthop Res. 2017;35:1774–1783
68. Wang B, Hao Y, Pu F, Jiang W, Shao Z. Computer-aided designed, three dimensional-printed hemipelvic prosthesis for peri-acetabular malignant bone tumour. Int Orthop. 2018;42:687–694

Chapter 10
Incidence, Complications, and Novel Treatment Strategies: Pediatric Spinal Surgery and Management

Hannah Gibbs, John F. Lovejoy III, and Ryan Ilgenfritz

Abstract Postoperative spine infections are common in the United States, complicating approximately 300,000 to 500,000 surgeries per year, with estimated costs of $1.6 billion. As spinal surgeries become more common, spinal instrumentation infection rates are only expected to rise, and as such, surgical site infections will continue to place tremendous economic and social burdens on both families and the healthcare system. A variety of spinal procedures are performed in the pediatric population, with posterior spinal fusion for adolescent idiopathic scoliosis being the most common. This chapter describes the common surgical approaches used in the treatment of pediatric spinal conditions. It also reports on the incidence and epidemiology of spinal infections, their complications, microbiology, presentation and diagnosis, as well as the techniques used to minimize contamination intraoperatively. Although extensive basic and clinical research efforts have occurred, little evidence and guidance exists for the management of spinal infections, especially in the pediatric population. As such, this chapter presents methods used in the management of surgical site infections and their efficacy while also highlighting novel and emerging treatment approaches that hold promise for the future.

Keywords Spinal surgery · Spine · Pediatric · Infection · Implants · Wound drains · Vacuum-assisted closure · Management · Novel treatments

H. Gibbs
College of Medicine, University of Central Florida, Orlando, FL, USA

J. F. Lovejoy III (✉)
College of Medicine, University of Central Florida, Orlando, FL, USA

Department of Orthopaedics, Sports Medicine and Physical Medicine and Rehabilitation, Nemours Children's Hospital, Orlando, FL, USA
e-mail: john.lovejoy@nemours.org

R. Ilgenfritz
Department of Orthopaedics, Sports Medicine and Physical Medicine and Rehabilitation, Nemours Children's Hospital, Orlando, FL, USA

10.1 Introduction

Postoperative spine infections are common in the United States, complicating approximately 300,000–500,000 surgeries per year, with estimated costs of $1.6 billion [1]. Preventative measures vary among institutions, and there is no real standard approach in the prevention and management of surgical site infection (SSI).

10.2 Incidence and Epidemiology

The overall incidence of spinal instrumentation infections in the pediatric population ranges from 0.5% to 20%, depending on surgical indication. There are a variety of spinal procedures performed in the pediatric population, with posterior spinal fusion for adolescent idiopathic scoliosis (AIS) being the most common. The overall incidence of scoliosis is about 2–3% of the population with the majority of curves managed conservatively with bracing. Surgical management is needed only in those with curves >50° [2]. Complications following spinal instrumentation surgery, such as superficial and deep wound infections, have rates that range from 0.5% to 4.3% in patients with AIS [3–7]. Recent data from the Scoliosis Research Society Committee showed an infection rate of 2.6% out of a total 20, 424 cases [8], although most of this cohort consisted of patients with AIS. Neuromuscular patients have higher rates of infections, of about 8–24% [3, 9–18], likely due to longer surgical times, poor bowel and bladder control, frequent urinary tract infections, cognitive impairment, malnutrition, and previous spine surgeries [15, 19–21]. Additionally, congenital scoliosis patients have reported infection rates of 2.2% [8]. Of importance, morbidity and mortality rates are highest in patients with neuromuscular scoliosis (19.9% and 0.34%, respectively) and congenital scoliosis (10.6% and 0.30%, respectively), compared to 6.3% and 0.02% respectively, in idiopathic scoliosis [22]. Patient characteristics and procedure-related variables have effects on SSI rates.

Surgical site infections place tremendous economic and social burdens on both families and the healthcare system. The mean hospital charge to a patient with a spine SSI is $154,537 but ranges between $26,977 and $961,722 [23]. Patients are typically hospitalized for an average of 29 days with chronic infections (>3 months) [23] and receive intravenous (IV) antibiotics with multiple surgeries and, in the unfortunate cases, implant removal [3, 23, 24]. In addition to the financial burden, patients and families deal with significant social stressors. Children and adolescents must miss school, parents must take time from work to care for them, and psychological stressors strain the family dynamic. Thus, prevention and management of spine SSI has been a recent focus in literature, although consensus and standardization are relatively sparse in the treatment of children and adolescents.

10.3   Common Surgeries and Approaches

10.3.1   Scoliosis

Scoliosis is characterized as lateral curvature of the spine, with a Cobb angle (angle between tilted vertebrae) greater than 10°. The etiology is multifactorial and unclear, but a genetic predisposition likely exists [25]. Scoliosis is classified according to age: infantile 0–3 years, juvenile 4–10 years, adolescent 11–17 years, and adult ≥18 years [26]. The most critical factor in determining natural history is age, as the younger a patient the more likely the curve will progress due to greater growth potential. For curves 25°–45°, bracing is considered first-line management. For curves 50° or more, surgical management via a posterior spinal fusion is recommended [26]. The primary goal of surgery is to impede the curve's progression, as curves >90° may cause significant pulmonary function limitations [27].

The surgery for correction is performed primarily from a posterior approach with the patient lying prone on the operating table. Hips are flexed to about 20°, and knees and legs are slightly flexed and elevated with pillows [28]. The abdomen is kept free to facilitate venous return. Throughout the entire procedure, electromyography neuromonitoring is attached to the patient to monitor for nerve root and sphincter functioning [29]. To surgically correct the spine, a midline incision is made, and facets are exposed via deep dissection. Facetectomies are performed until level with the transverse processes [23]. With the use of fluoroscopy, electromyography, and probe visualization technology, convex screws are visualized and drilled into the pedicles. Rods are then measured and set into pedicle screws loosely. Once the rods are set, the screws are tightened, and the rods undergo derotation to correct spinal deformity. The final step is copious irrigation and debridement of ischemic musculature (if any). Pre- and postoperative radiographs are shown below (Figs. 10.1a and 10.1b). Although less common, an anterior approach can be used for lumbar and thoracolumbar spinal fusions. In this case, the patient is placed in the lateral decubitus position with scoliosis curve facing upward [30].

10.3.2   Spondylolysis and Spondylolisthesis

Spondylolysis is a stress fracture in the pars interarticularis of the spine, whereas spondylolisthesis is a forward slip of one vertebra on its adjacent segment [31, 32]. The primary diagnosis is made by radiographs and may be confirmed with an MRI of the spine. Being that most cases are asymptomatic, first-line treatment is conservative bracing and monitoring. Symptomatic spondylolisthesis cases may require direct surgical repair from the posterior approach and, like scoliosis management, consists of a reduction and fusion. The procedure consists of pedicle screw combinations and compression wiring between the spinous and transverse processes [33–36].
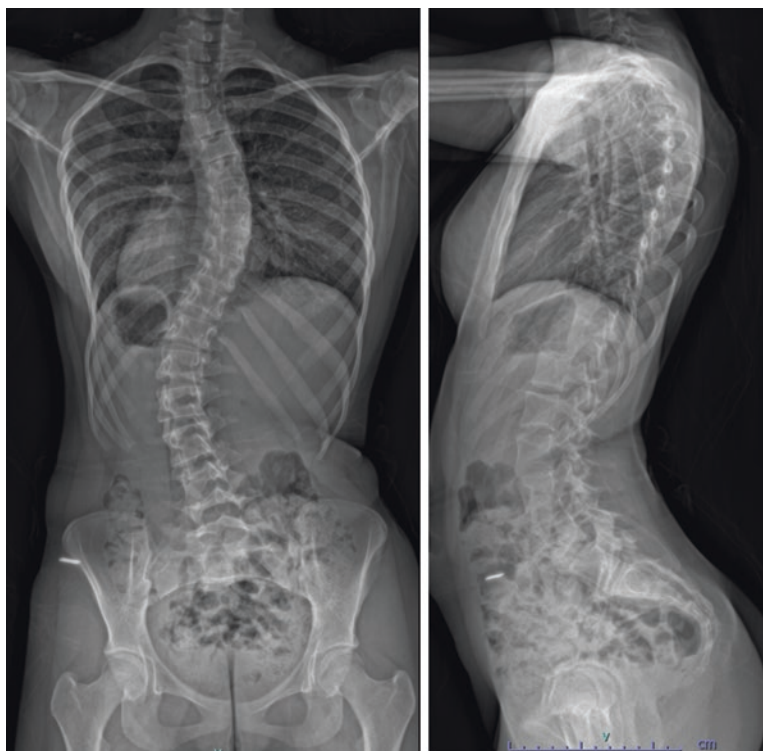
Fig. 10.1a PA and lateral scoliosis preoperative radiographs

10.3.3 Cervical Spine Instability

Cervical spine instability may present in otherwise healthy children, such as in the case of os odontoideum, or secondary to underlying conditions, such as Down syndrome or Klippel Feil syndrome [37]. Os odontoideum is when the upper portion of the dens (odontoid) separates from the base typically due to a nonunion fracture [37]. Most believe it is caused by unrecognized childhood trauma that fails to heal due to lack of blood supply or immobilization; however others believe the condition is congenital [38, 39].Neurologic deficits may develop as the os translates posteriorly it may impinge on the spinal cord. Patients with Klippel Feil syndrome have characteristic features including short, broad necks, restricted cervical motion, and low hairlines [37]. Patients with Down syndrome can have cervical instability due to congenital abnormalities of the cervical spine and ligamentous laxity. Atlantoaxial (C1-C2) subluxations and occipital-cervical instability are common conditions in this population. Last, cervical spine anomalies, such as atlantooccipital anomalies, increase C1-C2 instability and may lead to devastating outcomes if not surgically addressed. In severe presentations of cervical spine instability, i.e., those with cervical myelopathy and neurological symptoms, definitive management includes

Fig. 10.1b PA and lateral postoperative scoliosis radiographs

options such as a posterior spinal fusion of C1-C2, using the Gallie technique [37, 40] or a combined anterior posterior fusion.

For the Gallie technique, the patient lies prone, and an incision is made from the midline extending from the base of the skull to the C4 spinous process [41]. After deep dissection of the C1 and C2 vertebrae, a flexible double wire (18–22 gauge) is passed beneath the arch of C1 inferiorly to superiorly and bent back on itself to form a smooth lip. Bone graft, taken from corticocancellous bone of the iliac crest, is then constructed over the lamina of C1 and C2, and the loop of wire is fastened from the arch of C1, over the graft, around the base of C2.

10.3.4 Disc Herniations

Although rare in the pediatric population (1% incidence in those under 18) [42, 43], lumbar disk herniations can occur in children primarily due to trauma, and 95% of cases are at the L4-L5 or L5-S1 region [44–47]. Initial complaints are often pain with sciatica, and a magnetic resonance imaging (MRI) study can confirm the diagnosis. First-line management is nonoperative, but patients who fail this or have

significant neurologic symptoms such as extremity weaknesss should undergo surgical repair. The posterior approach is most common in these procedures, and it consists of an open discectomy to relieve the herniation of the nucleus pulposus into the spinal canal. Pain relief is present immediately following surgery.

10.4   Complications

As with any type of surgery, complications have the potential to occur and negatively affect the clinical outcome. Postoperative wound infections continue to be one of the most common complications following spine surgery. Identifying risk factors and implementing guidelines for preoperative, perioperative, and postoperative measures is integral in preventing postoperative infections. However, most of the available literature is limited by a retrospective study design, making it challenging to eliminate confounding variables and accurately identify factors in pediatric spine instrumentation infections. But through extensive research and systematic reviews [5, 48, 49], common risk factors for pediatric spinal instrumentation infections are recognized and can be stratified according to patient risk factors and procedure-related risk factors (Table 10.1). Patient-specific risk factors include prior spine surgery, age >10, American Society of Anesthesiology score >2 [5], neuromuscular conditions (cerebral palsy, myelomeningocele), urinary and bowel incontinence, obesity, recurrent UTIs, and malnutrition [48]. Procedure-related risk factors include perioperative antibiotic prophylaxis, blood loss and transfusions, the number of spinal levels fused, fusion to pelvis, implant prominence, use of allograft, and increased operative times [48]. Glotzbecker et al. [48] report the most important factors that increase the risk of SSIs are:

1. The inappropriate use of antibiotic prophylaxis
2. Increased implant prominence
3. Blood loss and transfusions
4. First-generation stainless steel implants
5. Number of spinal levels fused

Table 10.1 Spine surgical site infection risk factors [48, 49]

Pediatric patient risk factors	Procedure-related risk factors
Age >10	Perioperative antimicrobial prophylaxis
Prior spine surgery	Blood loss and transfusions
American Society of Anesthesiology score >2 (ASA)	Number of spinal levels fused
Obesity	Spinal fusion to the pelvis
Neuromuscular scoliosis	Use of allograft
Complex medical comorbidities (cerebral palsy, myelomeningocele)	Increased operative time
Urinary and bowel incontinence	Implant prominence
Recurrent UTIs	First-generation stainless steel implants
Malnutrition	

6. Fusion extension to the pelvis
7. Prolonged operative time

An additional study found a significantly increased risk for spinal instrumentation infections with inappropriate antibiotic use, neuromuscular scoliosis, number of postoperative hospital days, and rigid instrumentation, in the development of surgical site infections [50].

Complex medical conditions such as cerebral palsy, myelomeningoceles, and myopathies, are associated with increased rates of SSI likely due to weakened patient immune systems, urinary and bowel incontinence, and poor skin quality surrounding the lesion. Urinary and fecal incontinence leads to increased risk of infection with Gram-negative organisms due to direct spread of urinary and GI tract organisms [51], such as *Bacteroides fragilis* and *Escherichia coli* [52]. However, Mistovitch et al. [49] argue incontinence itself cannot be an isolated risk factor, as incontinence is present along with many other potential factors in neuromuscular scoliosis patients. Inappropriate antibiotic use, defined as incorrect dosage, antibiotic choice, or dose timing, was shown as a significant risk factor in the cause of SSI due to inadequate protection against pathogens [5]. Fusing more than ten vertebrae levels was associated with an increased SSI risk [6]. Pelvic fixation is a technique used to stabilize the spine and promote lumbosacral arthrodesis [53]. Six different studies have shown an increased likelihood for spinal infections with fixation of implants to the pelvis or sacrum due to the proximity to the bladder and bowels [12, 20, 51, 54–56].

The use of an allograft, or donor tissue graft, also increases the risk of developing spine instrumentation infections [15, 57]. One study found 46% of allografts to be contaminated with bacteria including *Pseudomonas aeruginosa, Staphylococcus epidermidis,* and others, prior to surgical implantation [58], likely caused by intraoperative preparation techniques. Patients are least likely to develop infections when bone autografts (host tissue graft) are used [59]. However, strong evidence reports that the use of ceramic allografts does not increase infection risk compared to autografts [60]. Lastly, the use of first-generation stainless steel rods are associated with an increased delayed (>1 year) SSI risk [61, 62] and challenge clearance of pathogens [63] due to the development of more extensive biofilms on steel rods in comparison to titanium [64]. Some advocate for the use of titanium rods as they offer enhanced protection against delayed SSIs and the production of biofilms [61, 64].

Deep spinal instrumentation infections are difficult to clear and may compromise correction of the deformity if the implant is removed [17]. Prolonged infections that do not clear can lead to serious complications such as vertebral osteomyelitis, sepsis, and neurologic deficits [17]. In more severe cases, the infection may be life-threatening. Another common complication of a postoperative infection is pseudoarthrosis, or failure of the spine to fuse after a fusion procedure is performed [65, 66]. When infections are suspected, careful intraoperative inspection of the fusion mass is necessary for detecting of pseudoarthrosis [15].

In addition to SSI, other rarer complications may occur in pediatric spine surgeries. Raemes et al. [22] report complications such as new-onset neurologic deficits, peripheral nerve deficits, pulmonary (not embolism) deficits, dural tears, implant failure, epidural hematomas, nonfatal hematologic deficits, deep venous thrombosis, SIADH, and vision deficits in pediatric patients who underwent spinal surgery.

10.5 Microbiology

Early detection of causative pathogenic organisms is vital in the management of postoperative spine infections. However, data is solely based on retrospective studies, limiting the accuracy and control of confounders. Cultures should be grown prior to the administration of antibiotics. Superficial wound cultures often have very low yields and rarely provide positive bacterial cultures. However, deep postoperative wound cultures yield pathogens in most (70–92%) cases of spine SSI [56, 67, 68], allowing for proper antibiotic therapy of choice. Regardless, pathogens can be stratified according to time elapsed since surgery and the initial surgical indications. The literature varies in defining early and late infections, but in general, an early infection is considered less than 90 days following the initial surgery, and a late infection is defined as greater than or equal to 90 days [23, 69]. Most studies report early infections being more common, with one reporting 67% of infections presenting within the first month and 90% within the first 6 months following surgery [56]. Earlier infections are more likely secondary to highly virulent pathogens, such as *Staphylococcus aureus* and Gram-negative enteric bacteria. Low virulent pathogens such as coagulase-negative staphylococci (CoNS), anaerobes, *Propionibacterium* spp., and *Enterococcus* spp. are more often found in later infections, as it takes time for these bacteria to form biofilms at which point they become extremely resistant to antibiotic therapy [70, 71]. Thus, low virulence and late infections are difficult to treat with medical therapy alone and typically require prompt removal of hardware for effective treatment [24, 69].

For idiopathic spinal surgery cases, Gram-positive organisms are the most common pathogens isolated, accounting for up to 87% of positive cultures [72, 73]. Methicillin-sensitive *Staphylococcus aureus (MSSA)* and CoNS are the most common organisms isolated in this cohort, composing 81% of all infections [72]. Methicillin-resistant *S. aureus (MRSA)* is rarer than MSSA, with a reported rate as low as 1.4% in the literature [72, 74]; however, this rate varies based on geographic location and the prevalence of MRSA in the community. It is important to note, the vast majority of organisms cultured from idiopathic spine infections were bacteria commensal to the skin [56]. The data compiled from studies of pediatric spinal instrumentation infections can be found in Table 10.2.

However, non-idiopathic scoliosis (neuromuscular and congenital scoliosis) patients have a higher prevalence of Gram-negative pathogens such as *Enterobacter* spp., *Escherichia coli, Pseudomonas, Actinobacter,* and *Proteus mirabilis* [15, 56, 75] and may be implicated in up to 57% of postoperative infections [56, 72].

Table 10.2 Microbiology of surgical site infections with spinal instrumentation in pediatric patients

Microorganisms[a]	Total infections	Idiopathic scoliosis	Non-idiopathic scoliosis	Early (<3 months)	Late (≥3 months)
Staphylococcus aureus	33.9%	25.6%	36.8%	31.4%	11.6%
Coagulase-negative *Staphylococcus*	18.1%	20.7%	14.0%	7.1%	18.8%
Streptococcus spp.	3.3%	1.2%	4.7%	1.5%	0.9%
Enterococcus faecalis	5.3%	6.1%	5.7%	7.1%	2.7%
Pseudomonas aeruginosa	5.3%	3.7%	6.7%	6.6%	1.8%
Enterobacteriaceae[b]	15.6%	–	20.7%	7.1%	0.9%
Propionibacterium acnes	5.6%	30.5%	2.6%	4.6%	41.1%
Anaerobes[c]	2.8%	7.3%	2.6%	5.1%	1.8%
Candida albicans	0.7%	–	0.5%	2.0%	–
Polymicrobial infection	7.4%	5.2%	5.2%	15.2%	7.1%
Other	2.1%	3.7%	0.5%	12.2%	13.4%

[a]Categories are not mutually exclusive

[b]*Enterobacteriaceae* includes *Enterobacter* spp., *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis*

[c]Excluding *Propionibacterium* species [3, 7, 15, 24, 57, 68, 72–74, 76–81]

There is a threefold increased risk of developing Gram-negative infections among patients with neuromuscular scoliosis [72], likely due to skin contamination with stool flora or direct inoculation of urine or stool to the wound [3, 57]. In comparison to monomicrobial Gram-positive infections, Gram-negative infections are more likely to be polymicrobial [15]. This data supports the necessity for Gram-negative prophylaxis before and during operations in those patients with non-idiopathic scoliosis.

Anaerobic species have also been implicated in surgical spinal infections, although with less frequency. Anaerobic species identified include *Peptostreptococcus* spp., *Propionibacterium (P. acnes), Finegoldia magna* [82]*, Bacteroides, Actinomyces,* and other Gram-positive anaerobic cocci [3, 24, 56, 72, 74, 77]. Infections with anaerobes are typically polymicrobial and are commonly identified in non-idiopathic scoliosis cases. *Propionibacterium acnes* is the only anaerobic bacteria that has been isolated monomicrobially and plays a significant role in late-onset spinal infections [69, 72] with a reported prevalence of 54% of cases in one study [77]. Since *P. acnes* infections are usually delayed >1 year and the presentation is subtle with no systemic symptoms, clinicians should have a high index of suspicion for this pathogen in late infections [62, 77]. Given the high prevalence of *P. acnes* in late-onset infections, some suggest evaluating for acne and possibly even a referral to dermatology, prior to scoliosis surgery in adolescents.

Although very rare in the literature, spinal instrumentation infections may be caused by fungi such as *Candida albicans, Mycoplasma hominis,* and non-tuberculosis *Mycobacterium* spp. [15, 77]. These pathogens were shown to cause more subacute infections and were successfully treated with irrigation and debridement alone [15].

The gold standard method in isolating pathogens is via intraoperative tissue culturing [83]; however, this method is not always consistent in identifying microorganisms. In two recent studies, 2.6–18% of swabs yielded negative cultures and the pathogen was not identified [56, 68]. Utilizing alternative methods such as lock analysis, confocal microscopy, or polymerase chain reaction (PCR) may enhance the reliability of identification [84]. Lock analysis is carried out by removing a small part of the implant during surgery, sterilely dividing it into smaller pieces, dipping the largest fragment into thioglycolate or brain-heart medium, and then culturing it on an agar plate for 8 days. Confocal microscopy uses a focal laser and fluorescence optics to investigate pathogens, and PCR utilizes 16s primers to detect sequences of a genome, enhancing accuracy in diagnosis and therapy [84]. These molecular methods have not yet been tested in pediatric orthopedic infections, but 16s PCR has shown promising results in adult prosthetic joint infections, accurately detecting up to 90% of pathogens [85]. For now, the best diagnostic test for pediatric spinal instrumentation infections is a combined swab of the implant and a sample of tissue directly in contact with the implant [84].

10.6 Minimizing Wound Contamination Preoperatively

The cause of spinal instrumentation infections is still poorly understood; thus, addressing pre-, intra-, and postoperative practices are imperative in reducing occurrences. Initially, skin preparation solutions, such as ChloraPrep® (2% chlorhexidine gluconate and 70% isopropyl alcohol) povidone-iodine (Betadine®), and DuraPrep® (0.7% iodine and 74% isopropyl alcohol) [86], should be used by the patient the night prior to surgery to minimize skin contamination [72]. Data is conflicting regarding which solution is most efficacious in reducing SSIs. A recent study in adults compared the effectiveness of ChloraPrep® versus povidone-iodine application and found ChloraPrep® to be superior in decreasing the risk of developing both superficial and deep SSIs [87]. However, Boston et al. [88] found povidone-iodine significantly decreased the risk of infection ($p < 0.001$), compared to other antiseptic agents. Lastly, one study reports no difference between antiseptics in reducing risk of infections [86]. The antiseptic of choice is ultimately at the discretion of the surgeon, but regardless of choice, patients should wash with it the night prior to surgery.

Preoperative patient education is also recommended to reduce infection risk postoperatively. Members of the Pediatric Orthopaedic Society of North America (POSNA) who were surveyed on preoperative practices reported 35% used patient

education sessions and handouts prior to surgery [63]. In fact, the Best Practice Guidelines (BPG) for High-Risk Pediatric Spine Surgery recommend patient education sheets prior to surgery as one of their 14 practices in decreasing SSI risk [89]. Other optional measures include obtaining preoperative urine cultures, labs (nutritional assessment), and clipping hair near the surgical site [89].

The presence of a UTI is another modifiable risk factor in the development of SSI [90, 91]; thus, acquiring preoperative urine cultures allows for the identification of possible pathogens and the chance to match sensitivities with targeted antibiotic prophylaxis. Hatlen et al. [90] reported two-thirds of patients with positive preoperative urine cultures later developed SSI with the same organism detected in the urine, further supporting this practice. Malnutrition has varying definitions in literature, the most common being total lymphocyte count <1500 cells/mm and preoperative albumin <3.5 g/dL [19]. There is strong evidence in adult populations regarding malnutrition and increased SSI risk, but this association is not as well demonstrated in the pediatric population, with one study reporting no association [20]. Nevertheless, the BPG recommends the use of preoperative nutritional assessments based on the findings of two studies in which investigators found increased risk for SSIs in malnourished children [19, 90]. Additionally, clipping hair around the surgical site is preferred over shaving [89].

In addition to the prior measures, a vital aspect of preoperative management is antibiotic prophylaxis, which is most effective when administered 30–60 min prior to surgery [20, 89]. Failure to give IV antibiotics within 60 min before incision time has been shown to increase the SSI risk in pediatric patients [5, 6, 20]. The American Academy of Orthopaedic Surgery considers perioperative IV cefazolin as first-line and standard of care in prevention of SSIs with orthopedic procedures [89]; however, recent studies challenge this practice and advocate for broader coverage, especially in subpopulations with non-idiopathic scoliosis [3, 5, 15, 20, 56]. Non-idiopathic scoliosis patients, such as neuromuscular scoliosis patients, are usually incontinent, leaving them more susceptible to Gram-negative infections. A study conducted in adults with chronic urinary tract colonization found those who were treated with individualized urine culture antibiotic prophylaxis developed less Gram-negative infections than those who received the standard IV cefazolin ($p = 0.039$) [92]. Hence Gram-negative coverage, in addition to IV cefazolin, is recommended in high-risk populations, such as those with neuromuscular scoliosis and chronic urinary tract colonization [93].

The final recommendation although controversial in literature is to perform preoperative nasal swabs for MRSA. The Best Practice Guidelines do not recommend performing the test with a 74% consensus disagreeing with the preoperative screen [89]. But other studies advocate for its use, as they found screening allowed for adjustment of the preoperative antibiotic agent, to avoid infections with MRSA pathogens, and antibiotic resistance [94].

10.7 Minimizing Wound Contamination Intraoperatively and Postoperatively

In addition to preoperative preventative measures, there are many perioperative efforts to minimize wound contamination. Prepping the surgical site with Ioban® antimicrobial draping is one of the first measures proven to prevent infections. A recent survey of pediatric orthopedic surgeons reported 62.9% of surgeons use Ioban® draping, after prepping the skin with an antiseptic [75]. With respect to the operating room (OR), most hospitals limit scrub wear outside of the facilities and limit OR traffic (both before and during surgeries) [75]. Some even utilize a "terminal sterilization" procedure that allows extra time between operations for sterilization following infection cases. Multiple studies have demonstrated the negative impact OR traffic has on orthopedic implant surgeries and surgical site infection rates [95–97]. Infection rates in total hip arthroplasty and total knee arthroplasty were recorded at 9% and 16.7%, respectively, prior to enforcing interventions such as limiting door openings and decreasing the amount of people in the OR during surgery; after making these changes, Borst et al. report a significant decrease in infection rates [96]. The BPG recommends limiting OR access, especially during scoliosis surgeries [89]. These practices ensure the safety of staff, aid in the maintenance of the sterile field, and limit patients from outside exposures. Despite some facilities using ultraviolet light for additional sterilization, the BPG advises against its use in the OR [89].

As far as the type of implant used in procedures, titanium rods have demonstrated superiority in decreasing the risk of infection compared to stainless steel rods [61, 62, 77], likely because titanium rods require increased concentrations of bacteria for colonization to occur. Patients with titanium instrumentation may also undergo an MRI, imaging that no other rod confers [98]. When possible, the use of titanium over stainless steel is recommended.

Intraoperative wound irrigation is necessary after spinal instrumentation is implanted. There are many options when it comes to irrigation, including irrigation solutions and bulb versus pulse lavage technique. Saline, bacitracin, and dilute povidone-iodine may be used as irrigation solutions. The choice is dependent upon the surgeon's preference as the literature varies on the most superior solution. Irrigation with povidone-iodine was shown to be more efficacious in reducing the risk of infection on spinal instrumentation procedures when compared to normal saline [99, 100], but the local administration of povidone-iodine may negatively affect bone growth as it has been shown to inhibit osteoblast proliferation [101], as well as cause nephrotoxicity [102].

Bone graft antibiotics may be used to decrease the proliferation of microorganisms upon implantation. To date, only one study has evaluated the use of antibiotics in grafts in the pediatric population [11]. Borkhuu et al. [11] found a decreased incidence of deep spine infections with the use of gentamicin-treated corticocancellous bone graft (3.2%) when compared to non-treated bone allograft (15.2%) ($p = 0.003$). Bone allograft may be used in procedures as it has low risks of

infectious disease transmission and low complications rates and has demonstrated great success in both neuromuscular and idiopathic scoliosis patients [103–106]. A previous concern with allograft was an increased risk of infection when compared to autograft, but as shown in this study, porous allograft bone may be freeze-dried with added antibiotics to mitigate this elevated risk. Other studies also of allograft utilization have no difference in rates of infection [32, 107]. Thus, the use of antibiotic-infused bone allograft may be a beneficial intraoperative measure in prevention of wound contamination.

In addition to graft antibiotics, vancomycin powder applied over the open wound is a common intraoperative practice in spinal fusion procedures. Sweet et al. [108] studied the effects of intrawound vancomycin in adults and found a decreased infection rate from 2.6% to 0.2% in those that used the vancomycin powder. Other studies in literature also demonstrate the beneficial effects of vancomycin in reducing infection risk [109, 110]. The local administration of vancomycin allows for higher concentrations without the adverse effects seen systemically. To eradicate methicillin-resistant *Staphylococcus aureus*, the minimum inhibitory concentration (MIC) of vancomycin must be >1 μg/mL [111, 112]. A recent study found that when local intrawound vancomycin was given, concentrations on day 0 were thousand-fold more than the MIC (1457 μg/mL) and were still elevated on postoperative day 3 (128 μg/mL) [108]. In addition, vancomycin is not well absorbed into the bloodstream when administered locally unlike other topical antibiotics (aminoglycosides) [108]. There is strong evidence supporting the safe use of intrawound vancomycin in the pediatric population, as studies have shown no increase in serum vancomycin levels and no change in creatinine when used [113]. Another valid concern is vancomycin's probable inhibitory effect on osteoblasts and impairment of bone regeneration, but Philp et al. [114] found vancomycin's inhibition to be minute. Despite these strong recommendations, one study reports no difference in spinal instrumentation infections with the use of powdered vancomycin versus standard perioperative IV antibiotic prophylaxis in a cohort of 907 patients [115]. In summary, the Best Practice Guideline's and other studies support the use of surgical site vancomycin in the prevention of wound contamination, especially in high-risk pediatric patients [89].

Prolonged operative time is also shown to be positively correlated with spinal instrumentation infections. Since the procedures of the spine are typically deep and extensive in length, the closure of the muscle, fascia, and skin contributes notably to operative time. No difference in spinal instrumentation infection risk has been found with different closure methods, but significantly decreased wound closure time has been demonstrated using barbed and zipper sutures [116–118]. Literature for orthopedic knee arthroplasty has demonstrated more rapid wound closure using bidirectional barbed sutures [119–121]. Mansour et al. [117] tested this theory in the case of AIS and posterior spinal fusion by comparing closure times using traditional layered interrupted suture versus barbed suture and found wound closure time with the barbed suture to be significantly less (12.5 minutes less), than with traditional sutures ($p < 0.001$). Less operative time may possibly decrease the risk of SSI but affirmatively results in diminished hospital costs per case. Mansour and

investigators [117] estimate rapid closure rates saving the hospital an estimated $884.60 per case. Thus, barbed suture may reduce hospitals' significant yearly costs worldwide. In the same light, when zipper sutures and traditional Monocryl 4-0 suture were compared, the zipper closure method took significantly less time (45.3 s vs. 540.5 s, respectively) ($p < 0.001$) [116]. Patient satisfaction and cosmetic results were similar in both groups as well [116], indicating the zipper as a safe, effective, faster, and a satisfactory option for wound closure. Lastly, cyanoacrylate liquid (Integuseal® sealant) may be used as a microbial sealer at the end of surgery. Although there were no reported adverse effects or sensitivity reactions, there was no significant correlation found between the use of Integuseal® and prevention of postoperative infection occurrences ($p = 0.096$) [118].

Debate exists regarding the duration of postoperative antibiotic prophylaxis. Fear of infections and complications urges some to continue use for 24–48 h postoperatively [122, 123]. Prolonged antibiotic courses may cause unfavorable adverse effects, leading to more harm than good. One study found no significant difference in spinal instrumentation infections when antibiotics were given 24 h versus 72 h in adults who underwent posterior spinal fusion [124]. Correspondingly there was no difference in AIS posterior spinal fusion patients when antibiotics were given until drain removal (3–5 days) versus two postoperative doses ($p = 1.0$) [125]. Thus, the general consensus is the length of postoperative antibiotics is not associated with decreased SSI risk.

Finally, to minimize wound contamination postoperatively, the Best Practice Guidelines suggest reducing postoperative dressing changes, especially in those who are urinary and fecal incontinent to provide a barrier and seal [89]. A recent study found when restricting dressing changes for 5 days or more postoperatively, posterior spinal fusion SSIs significantly decreased from 3.9% to 0.93% ($p < 0.0001$) [126], as the dressing likely provided protection against nosocomial pathogens. Despite significant findings with duration and minimization of changes, there was no reported difference in infection risk between silver-impregnated dressings and standard gauze in pediatric patients [127]. Preoperative, intraoperative, and postoperative measures are summarized in Table 10.3.

10.8 Wound Drains

Wound drains are used to prevent the formation of hematomas and seromas [128]. Hematomas have the potential to delay wound healing, via increased wound tension. They may also increase the risk of postoperative infections [128, 129], and they have the potential to compress the spine, causing cauda equina or neurologic deficits [130]. The use of wound drains is controversial in the literature, notably in the pediatric spinal fusion population [131]. Some advantages of using a drain are the minimization of hematomas and seromas, therefore decreasing the risk of cord compression and infections, as the hematoma serves for bacterial breading ground [132]. However, wound drains may interfere with mobilization, become

Table 10.3 Measures taken to minimize surgical site infections in spine surgery Also under Intraoperative measures

Preoperative measures	Intraoperative measures	Postoperative measures
Chlorhexidine skin prep the night before surgery	IV cefazolin (clindamycin if allergic to cefazolin) prophylaxis within 60 min of incision time	Minimize dressing changes
Patient education sheets or teaching session	IV antibiotic (Gram-negative coverage) prophylaxis[a]	Postoperative antibiotic prophylaxis
Urine cultures	Limit operating room (OR) access during procedure	Dressing duration
Acne treatment	Ultraviolet lights are *not* recommended	
Clipping hair preferred over shaving	Intraoperative wound irrigation should be performed with normal saline	
Nutritional assessment (total lymphocyte count, pre-albumin)	Vancomycin powder should be utilized in bone graft or at the surgical site	

[a]High-risk patients: those with neuromuscular scoliosis, myopathies, and other non-idiopathic conditions [48, 49, 89]

contaminated, cause postoperative pain and anxiety, increase the risk of blood loss, and require higher levels of care [130, 132]. Multiple studies have demonstrated the increased risk of blood loss leading to postoperative anemia and the requirement of more blood transfusions in patients with drains when compared to patients with no drains [133, 134]. A recent study detected a significant difference in hemoglobin levels between drain and no drain patients, finding significantly less hemoglobin in drain patients ($p < 0.001$). Iatrogenic trauma may also occur with drain placement, and hospital stays are significantly longer because of pain and immobilization [130]. Blank et al. [135] reported improved postoperative wound healing and significantly fewer dressing saturations in AIS patients with wound drains. Another study in adults who underwent lumbar procedures and received wound drains was found to have no significant difference in postoperative infection rates, neurologic deficits, OR time, blood loss, hemoglobin or hematocrit levels, and length of hospital stay [136]. Meta-analyses that compiled data from drains and no drains reported a significant difference in the dressing saturation between groups ($p = 0.002$) [137]. There were no reported differences in wound infection, hematomas, or estimated blood loss.

Another issue with drains is the lack of standardization with drain practice patterns such as deep versus superficial drain placement, bulb versus wall suction, method of drain stabilization (tape, suture to skin), duration of placement, and anticipated drain outputs. In a cohort of 50 pediatric spine surgeons, 36 (72%) used drains following posterior spinal fusions, and 18 of them report doing so out of habit [132]. The other indications they reported for drain placement were excessive bleeding, presence of an open vertebral canal, revision case, and INR >1.2 (increased risk of bleeding). No clear indications or guidelines have been published regarding

wound drain utilization. Most of the surgeons in this study left the drain in for 48 h [132], which was similar to the average of 46 h recorded in a different study [130].

Despite these complications and ambiguities, most of the literature reports no effect of drains on the rate of SSI in the pediatric population [20, 51, 132, 138, 139]. To date, one study has demonstrated a protective effect of drains against spinal instrumentation infections [32]. Therefore, the overall consensus is use of drains neither increases nor decreases the risk of infection but may increase complications following surgery [140, 141]. Hence, wound drains are not routinely used in prevention of surgical spinal infections.

10.9 Wound Vacuum-Assisted Closure (VAC)

Wound vacuum-assisted closure (VAC) devices are very useful for wound healing by secondary intention or an open wound healing from the base upward. Designed initially for chronic soft tissue wounds, like decubitus ulcers, wound VACs are transitioning to the field of orthopedics [142]. VACs function by providing continuous subatmospheric pressures within the wound (−125 mmHg), which promotes angiogenesis and the formation of granulation tissue, drains edematous fluid, and provides barrier dressings throughout treatments [143]. VACs may be closed in primary wounds or may be used post irrigation and debridement of infected spinal tissue, with planned delayed wound healing.

To date, few reports on utilization of VACs with spine surgeries exist. These devices were first introduced to pediatric spine surgery with two medically complex patients who developed deep wound infections [144]. The first patient suffered from Hurler's disease and underwent a posterior spinal fusion to repair severe kyphosis. His deep wound infection was ultimately treated with a VAC system for 6 weeks by secondary closure (use of a split skin graft over the wound). This patient fully recovered without exchange or removal of the implant. The second patient had a chronic thoracic fistula following closure of spina bifida that was successfully treated with the wound VAC system for 10 weeks. Two additional studies looked at wound VAC closures for 1–2 weeks following deep wound spinal infections [145, 146]. Twenty-one/21 (100%) patients had complete infection resolution with the use of a wound VAC alone [145], whereas the other had resolution of the infection in 5/6 patients [146]. Neither study required removal of instrumentation. Lastly, van Rhee et al. [142] carried out a case study on six neuromuscular pediatric scoliosis patients who presented with deep wound infections following posterior spinal fusions. The VAC was changed three times per week either at home or in the hospital, and each patient was given adjunctive parenteral antibiotics for at least 6 weeks. After 6 weeks of treatment, ESR and CRP were trending down near normal, and after an average of 3 months, the wounds had healed by secondary intention with resolved infections [142]. The formation of granulation tissue over spinal instrumentation was the time-limiting factor in wound closure; however, removal of instrumentation was unnecessary in all six patients. These studies provide promising data and support the use

of wound VACs for wound healing by secondary intention, specifically following irrigation and debridement for wound infections.

Although rare, wound VACs may cause skin allergies, breakdown of the skin, and pain when exchanging the foam pads [142], although no complications were reported in van Rhee's six-patient case study. Lastly, wound VACs create an anaerobic environment and may harbor anaerobic bacterial infections. High suspicion and consistent culturing of the wound is recommended to avoid complications [147].

10.10   Clinical Presentation and Diagnosis

Making the diagnosis of spine infections requires clinical judgment since patients do not always manifest with apparent symptoms [83]. The most common presenting symptoms are wound drainage, back pain, and, although less common, fever and malaise [57]. In one cohort of spine SSI patients, 86.2% presented with wound drainage [74]. Generally, back pain and wound drainage are the most common presenting symptoms [57]. On physical exam, the wound may appear completely normal given the vast distance between superficial skin and deep layers of the wound [15], or wound dehiscence and sinus tracts may be present. If an infection is suspected, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) labs should be drawn. WBC count is a weak and unreliable predictor of infection, but acute-phase reactants are more useful despite inconsistency. Since labs are usually normal or slightly elevated, drawing conclusions may be challenging [148, 149]. ESR may be elevated for up to 6 weeks following surgery, whereas CRP levels typically normalize by 2 weeks. Thus, CRP is considered the most sensitive indicator in the detection of SSIs [150]. Blood cultures may also be used to guide antibiotic therapy, although false-negative rates are near 88% [148, 149]. Imaging modalities include X-rays, computed tomography scans, and ultrasound. Plain X-rays are typically useful in the early diagnosis of infection because changes are often not yet present [83].

As mentioned above, superficial swabs of the wound have low yields for culture; therefore deep swabs are preferred. The best way to establish a diagnosis is by aspirating the wound [15]. This is done by inserting an 18-gauge 3.5-inch needle through sterilely prepared skin, starting at the deep layer of the wound [15]. The clinician should advance the needle until they can feel the instrumentation [15]. If the deep layer's aspiration yields an unremarkable sample, aspiration of the superficial layer should occur. The diagnosis is confirmed with a positive culture result. Deep wound infections require surgical confirmation to identify infectious tissue in contact with the implant or fusion mass. Even if a hematoma or seroma exists, an infection may still be present and should not be ruled out. Any postoperative wound hematomas or wound drainage should alert clinicians to pursue further workup.

10.11 Management of Surgical Site Infections

Although extensive basic and clinical research efforts have occurred, little evidence and guidance exists for management of spinal infections, especially in the pediatric population. Not all infections are equal in severity or have the same level of involvement of the surgical site which creates challenges in managing infections [83]. Superficial infections are limited to the dermis and subcutaneous tissue without crossing the deep fascia [11]. Deep infections extend beyond the deep thoracodorsal or lumbodorsal fascia [11]. In general, antibiotic therapy alone is sufficient for the treatment of superficial spine infections and those without instrumentation; however, spinal instrumentation infections are difficult to clear as they offer an environment that harbors bacterial growth. In general, surgical debridement and antimicrobial therapy is agreed upon as the initial step in managing spine infections, but the treatment varies based on early-onset (<3 months) or late-onset infections (≥3 months). Persistent infections can require exchange of hardware, or once fusion has occurred, permanent removal of the implants.

10.11.1 Medical Management

Most of the recommendations for pediatric spine SSI are drawn from adult prosthetic joint infection literature. Generally, spinal instrumentation infections are best treated with implant removal, but this may not always be necessary or safe due to early occurrence after placement. The Infectious Diseases Society of America guidelines for the treatment of adult prosthetic joint infections recommend beginning with debridement, antibiotics, and implant removal (DAIR) [151]. Antibiotic therapy based on the organism and drug sensitivity profiles should be given parenterally for 2–6 weeks, followed by oral medication for 3–6 months or more [151]. Some studies recommend IV antimicrobial therapy for 8 weeks or more if the cultured organism is resistant, such as MRSA [137]. Kowalski and colleagues [69] demonstrated successful eradication in 28/30 patients that underwent DAIR management for early (<30 days) spine infections. They gave patients at least 2 weeks of parenteral antibiotic therapy and then transitioned to oral meds for 6 months or more. In this early onset-infection cohort, per os (PO) antibiotic therapy was associated with an increased probability of survival.

The optimal length of antibiotic therapy is patient and case dependent and varies throughout studies. Messenia and colleagues [74] followed 23 patients with spine infections and managed them with incision and drainage, pulsatile irrigation with normal saline, and treatment with antibiotics for a median of 131 days (42–597). Therapy was guided by resolution of symptoms and inflammatory markers. Eighteen of 23 patients were successfully treated with implant retention and antibiotic therapy alone, but the medical treatment did not come without consequences. Of the 23 patients, seven had adverse effects on prolonged medications, the most common

being nephrotoxicity, hepatotoxicity, and reversible neutropenia. All were reversible upon stopping the medications except for ototoxicity, which one patient developed after taking aminoglycosides.

The medication regimen should consist of pathogen-specific antimicrobial agents in combination with rifampin for staphylococcal infections [151]. The recommended regimen for Staph infections would be combination therapy of vancomycin and rifampin [151]. Other companion drugs include fluoroquinolones, minocycline, doxycycline, co-trimoxazole, or oral first-generation cephalosporins. Surgeons should alter therapy based on adverse effects, intolerances, allergies, and in vitro susceptibility. Recent studies report successful eradication of both staphylococcal and streptococcal infections, as well as other Gram-positive organisms with the use of rifampin [152]. If the infecting pathogen is Gram-negative, fluoroquinolones should be used as studies have demonstrated higher success rates with this therapy [153, 154]. Typically, the use of fluoroquinolones in pediatrics is discouraged due to antibiotic resistance and negative adverse effects, but the American Academy of Pediatrics (AAP) supports the use of fluoroquinolones for the treatment of multidrug-resistant infections where the use is deemed appropriate and no safe alternatives exist [155].

10.11.2   Surgical Management

The initial treatment for all spine instrumentation infections is irrigation with normal saline and debridement of ischemic or devitalized tissue [15]. Sponseller et al. [15] report successful eradication using this method against Gram-positive pathogens. The fusion mass should be explored carefully to evaluate for arthrodesis or pseudoarthrosis. Secondary measures such as wound VACs or closed suction drains may be used in adjunct with surgery. If the patient is septic or if muscle ischemia was found, the surgeon should not hesitate to leave the wound open until patient improves. Open wounds need special attention with appropriate debridement frequency and therapy. Considerations of implant removal, retention, debridement, and wound management are based on surgeon discretion.

After debridement, the wound may be closed primarily over a drain or left open to heal by secondary intention. Secondary intention wounds heal by the growth of granulation tissue covering the implant, deep to superficial. Sponseller et al. [15] recommend primary closure if adequate muscle can be mobilized and if the closure is done early on. Closing primarily over a drain following irrigation and debridement has shown to be highly successful in spine instrumentation infections [156]. But closure is complicated if paraspinal muscles are too stiff, and in these scenarios, local rotational muscle flaps may assist wound closure. The latissimus dorsi may be used to close upper lumbar and thoracolumbar wounds, whereas the trapezius may be used to close upper cervical and thoracic wounds [15]. One study used flaps composed of latissimus dorsi or gluteus maximus muscles to enhance blood supply

to the area and achieve appropriate wound closure and had excellent wound healing results [157].

Sponseller and colleagues [15] created clear indications for treatment in the event of pediatric spine instrumentation infections. With initial surgical exploration of the implant, as long as the muscle surrounding implant appears viable and the patient is stable, debridement and wound closure should occur. If extensive purulence or poor tissue quality and coverage is seen, the wound should be left open to heal by granulation over instrumentation (secondary intention). Last, if the wound continues to have purulent drainage even with multiple prior debridements and treatment, the implant should be removed.

Whether to retain, remove, or exchange the implant depends upon the timing of infection. Early-onset infections, as mentioned previously, typically heal with DAIR alone, with the primary goal being retention of the implant. However, late-onset infections, especially those that are deep, are almost always treated with surgical removal or exchange of implant [15] due to the risk of infection recurrence and failure of eradication with DAIR alone. Later-onset infections are caused by low virulence pathogens that have formed biofilms over the implants, making it extremely difficult to clear the resistant biofilm [70, 71]. Implant removal is also recommended if *Propionibacterium* spp. are isolated [158]. Ho et al. [24] argue the only way to completely eradicate late infections is through implant removal. Other studies report an almost 50% chance of infection recurrence without the removal of instrumentation [159], compared to a 10% chance with implant removal.

Instrumentation removal, either early or late, has a risk of causing progression of the spinal deformities [3, 7, 23, 24, 81], notably in earlier infections since the spine is less likely to be fused. There is varying literature on the degree of progression following removal, with some reporting 10° or more [160] to others >23° [3]. Regardless of infection timing, curve progression is likely to occur after implant removal [69, 160, 161]. A recent study evaluated curve progression in 21/42 AIS patients who required implant removal for instrumentation infections. Nineteen of 21 patients had an 11–20-degree increase in thoracic kyphosis, and 5/21 had >20-degree progression of the thoracic curve [160]. These patients with significant progression had greater thoracic and lumbar coronal curves prior to surgery, although, time from fusion to removal and reason for removal of the implant was not correlated with progression. Another study showed overall curve progression in implant removal to be 23° versus 2° in those who had implant retention [3]. On average, this cohort required two surgeries (range 1–9) for eradication. Other studies report lesser curve progression such as 10° in thoracic curve [24] and 6° in thoracic or 5° for lumbar curves [67]. Although a lesser degree of progression is seen in those >1 year from initial operation, progression of the curve is expected but unpredictable; therefore, if a later-onset deep infection exists, implant removal should occur despite progression for safety of the patient.

An additional complication of implant removal is pseudoarthrosis or failure of spinal fusion following a procedure. One study reports patients with implant removal having higher pseudoarthrosis rates compared to those with retention (38.1% to 0%, $p = 0.02$) [161]. However, other studies report pseudoarthrosis incidence in patients

with late instrumentation infections to range from 20% to 62% [162, 163]. Cahill and colleagues [3] reported of 13 patients that developed late infections and 7/13 later developed pseudoarthrosis. The average number of procedures required to manage pseudoarthrosis was 1.2. Although these studies demonstrate an association between late spinal infections and pseudoarthrosis, some authors challenge the ideal and ask, is spinal infection a risk factor in the development of pseudoarthrosis? Or is pseudoarthrosis a predisposing risk factor in the development of spinal infections [162]?

An additional option, although less common, is implant exchange in the management of instrumentation infections. Since implant removal is avoided early on, exchanging the prior implants with new ones may be a successful way to eradicate early infections. In a review of patients with chronic infections, ten patients underwent implant exchange and had better outcomes with curve progression with respect to those who underwent implant removal [67]. Another study using implant exchange for acute infections successfully cleared infections 76% of the time [63]. At the least, implant exchange from stainless steel to titanium rods is recommended in patients with stainless steel implants, as infections are more difficult to clear with stainless steel [63]. In summary, early infections should be managed with irrigation, debridement, implant retention, and prolonged antibiotic therapy, whereas late infections have better outcomes if managed with implant removal and antibiotic therapy.

10.11.3 Prognosis

Despite the significant financial and social burdens of postoperative spinal infections, once treated, patients have outcomes similar to those with normal postoperative courses. Management with irrigation, debridement, retention or removal of the implant, and prolonged antibiotic therapy has been shown to provide similar prognoses for patients without infections [164]. However, aggressive management is required to avoid the complications of sepsis, vertebral osteomyelitis, and loss of spinal correction.

10.12   Novel Treatments in Minimizing Surgical Spine Infections

As spinal surgeries become more common, spinal instrumentation infection rates are expected to rise; thus, earlier detection and better treatment methods are of great interest. Many authors have identified markers for early prediction of spine SSI [165–169]. Overall, decreased postoperative lymphocyte percentages, increased neutrophil to lymphocyte ratios, and increased neutrophil percentages were found to predict the development of SSIs [165–169]. Lymphocyte percentages ≤15.1% at 3–4 days postoperative and ≤19.1% on postoperative day 7 were associated with a

significantly increased risk of developing SSI after spinal decompression surgeries in adults [166]. Neutrophil to lymphocyte ratios of ≥3.21–3.87% were also early predictors in the development of spinal infections following adult decompression and instrumentation surgeries [165, 166]. Last, when the percentage of postoperative neutrophils exceeded 69% on days 6–7, significant increases in SSIs were seen in adult posterior lumbar and instrumentation surgeries [165, 167].

As mentioned, there is no gold standard for spine instrumentation infection management as there is for knee and hip arthroplasty. But promising results with the use of antibiotic-impregnated, permanently implanted polymethylmethacrylate (PMMA) cement in patients with deep spinal instrumentation infections were recently found to be efficacious in eradication [169]. In this specific study, the cement was infused with vancomycin and tobramycin. Ten patients with deep SSI were treated with one irrigation and debridement procedure and the addition of antibiotic-impregnated PMMA cement. None of the patients required implant removal at the average 64.4-month follow-up period. The cement is likely efficacious due to exothermic reactions that occur with cement solidification, thus increasing the permeability of biofilms and the susceptibility to antibiotics [169]. In addition, antibiotic-loaded polymethylmethacrylate (PMMA) cement is likely efficacious as it provides high local antibiotic concentrations [170].

Despite the rarity of spinal infections, a variety of preoperative, perioperative, and postoperative management strategies exist. More research is needed to evaluate clear management guidelines, especially in the pediatric population.

References

1. Martone WJ, Nichols RL. Recognition, prevention, surveillance, and management of surgical site infections: introduction to the problem and symposium overview. *Clin Infect Dis.* 2001;33 Suppl 2:S67–68. https://doi.org/10.1086/321859.
2. Montgomery F, Willner S. The natural history of idiopathic scoliosis. Incidence of treatment in 15 cohorts of children born between 1963 and 1977. *Spine (Phila Pa 1976).* 1997;22(7):772–774. https://doi.org/10.1097/00007632-199704010-00012.
3. Cahill PJ, Warnick DE, Lee MJ, et al. Infection after spinal fusion for pediatric spinal deformity: thirty years of experience at a single institution. *Spine (Phila Pa 1976).* 2010;35(12):1211–1217. https://doi.org/10.1097/BRS.0b013e3181c212d1.
4. Coe JD, Arlet V, Donaldson W, et al. Complications in spinal fusion for adolescent idiopathic scoliosis in the new millennium. A report of the Scoliosis Research Society Morbidity and Mortality Committee. *Spine (Phila Pa 1976).* 2006;31(3):345–349. https://doi.org/10.1097/01.brs.0000197188.76369.13.
5. Linam WM, Margolis PA, Staat MA, et al. Risk factors associated with surgical site infection after pediatric posterior spinal fusion procedure. *Infect Control Hosp Epidemiol.* 2009;30(2):109–116. https://doi.org/10.1086/593952.
6. Milstone AM, Maragakis LL, Townsend T, et al. Timing of preoperative antibiotic prophylaxis: a modifiable risk factor for deep surgical site infections after pediatric spinal fusion. *Pediatr Infect Dis J.* 2008;27(8):704–708. https://doi.org/10.1097/INF.0b013e31816fca72.

7. Rihn JA, Lee JY, Ward WT. Infection after the surgical treatment of adolescent idiopathic scoliosis: evaluation of the diagnosis, treatment, and impact on clinical outcomes. *Spine (Phila Pa 1976).* 2008;33(3):289–294. https://doi.org/10.1097/BRS.0b013e318162016e.

8. Smith JS, Fu KM, Polly DW, Jr., et al. Complication rates of three common spine procedures and rates of thromboembolism following spine surgery based on 108,419 procedures: a report from the Scoliosis Research Society Morbidity and Mortality Committee. *Spine (Phila Pa 1976).* 2010;35(24):2140–2149. https://doi.org/10.1097/BRS.0b013e3181cbc8e7.

9. Banit DM, Iwinski HJ, Jr., Talwalkar V, Johnson M. Posterior spinal fusion in paralytic scoliosis and myelomeningocele. *J Pediatr Orthop.* 2001;21(1):117–125. https://doi.org/10.1097/00004694-200101000-00023.

10. Benson ER, Thomson JD, Smith BG, Banta JV. Results and morbidity in a consecutive series of patients undergoing spinal fusion for neuromuscular scoliosis. *Spine (Phila Pa 1976).* 1998;23(21):2308–2317; discussion 2318. https://doi.org/10.1097/00007632-199811010-00012.

11. Borkhuu B, Borowski A, Shah SA, Littleton AG, Dabney KW, Miller F. Antibiotic-loaded allograft decreases the rate of acute deep wound infection after spinal fusion in cerebral palsy. *Spine (Phila Pa 1976).* 2008;33(21):2300–2304. https://doi.org/10.1097/BRS.0b013e31818786ff.

12. Geiger F, Parsch D, Carstens C. Complications of scoliosis surgery in children with myelomeningocele. *Eur Spine J.* 1999;8(1):22–26. https://doi.org/10.1007/s005860050122.

13. McMaster MJ. Anterior and posterior instrumentation and fusion of thoracolumbar scoliosis due to myelomeningocele. *J Bone Joint Surg Br.* 1987;69(1):20–25.

14. Osebold WR, Mayfield JK, Winter RB, Moe JH. Surgical treatment of paralytic scoliosis associated with myelomeningocele. *J Bone Joint Surg Am.* 1982;64(6):841–856.

15. Sponseller PD, LaPorte DM, Hungerford MW, Eck K, Bridwell KH, Lenke LG. Deep wound infections after neuromuscular scoliosis surgery: a multicenter study of risk factors and treatment outcomes. *Spine (Phila Pa 1976).* 2000;25(19):2461–2466. https://doi.org/10.1097/00007632-200010010-00007.

16. Stella G, Ascani E, Cervellati S, et al. Surgical treatment of scoliosis associated with myelomeningocele. *Eur J Pediatr Surg.* 1998;8 Suppl 1:22–25. https://doi.org/10.1055/s-2008-1071247.

17. Szöke G, Lipton G, Miller F, Dabney K. Wound infection after spinal fusion in children with cerebral palsy. *J Pediatr Orthop.* 1998;18(6):727–733.

18. Teli MG, Cinnella P, Vincitorio F, Lovi A, Grava G, Brayda-Bruno M. Spinal fusion with Cotrel-Dubousset instrumentation for neuropathic scoliosis in patients with cerebral palsy. *Spine (Phila Pa 1976).* 2006;31(14):E441–447. https://doi.org/10.1097/01.brs.0000221986.07992.fb.

19. Jevsevar DS, Karlin LI. The relationship between preoperative nutritional status and complications after an operation for scoliosis in patients who have cerebral palsy. *J Bone Joint Surg Am.* 1993;75(6):880–884. https://doi.org/10.2106/00004623-199306000-00008.

20. Labbé AC, Demers AM, Rodrigues R, Arlet V, Tanguay K, Moore DL. Surgical-site infection following spinal fusion: a case-control study in a children's hospital. *Infect Control Hosp Epidemiol.* 2003;24(8):591–595. https://doi.org/10.1086/502259.

21. Olsen MA, Mayfield J, Lauryssen C, et al. Risk factors for surgical site infection in spinal surgery. *J Neurosurg.* 2003;98(2 Suppl):149–155.

22. Reames DL, Smith JS, Fu KM, et al. Complications in the surgical treatment of 19,360 cases of pediatric scoliosis: a review of the Scoliosis Research Society Morbidity and Mortality database. *Spine (Phila Pa 1976).* 2011;36(18):1484–1491. https://doi.org/10.1097/BRS.0b013e3181f3a326.

23. Hedequist D, Haugen A, Hresko T, Emans J. Failure of attempted implant retention in spinal deformity delayed surgical site infections. *Spine (Phila Pa 1976).* 2009;34(1):60–64. https://doi.org/10.1097/BRS.0b013e31818ed75e.

24. Ho C, Skaggs DL, Weiss JM, Tolo VT. Management of infection after instrumented posterior spine fusion in pediatric scoliosis. *Spine (Phila Pa 1976).* 2007;32(24):2739–2744. https://doi.org/10.1097/BRS.0b013e31815a5a86.

25. Koop SE. Infantile and juvenile idiopathic scoliosis. *Orthop Clin North Am.* 1988;19(2):331–337.

26. Weinstein SL, Flynn JM. *Lovell and Winter's pediatric orthopaedics.* Lippincott Williams & Wilkins; 2013.

27. Pehrsson K, Bake B, Larsson S, Nachemson A. Lung function in adult idiopathic scoliosis: a 20 year follow up. *Thorax.* 1991;46(7):474–478. https://doi.org/10.1136/thx.46.7.474.

28. Hedequist D. Scoliosis Correction In: Saunders, ed. *Operative Techniques: Pediatric Orthopaedic Surgery* Elsevier; 2011:719–730.

29. Tan AHC, Lam KS, Lee EH. The treatment outcome of trigger thumb in children. *Journal of Pediatric Orthopaedics B.* 2002;11(3):256–259.

30. Karlin LI. Anterior Spinal Instrumentation and Fusion for Lumbar and Thoracolumbar Idiopathic Scoliosis In: Saunders, ed. *Operative Techniques: Pediatric Orthopaedic Surgery* Elsevier 2011:731–753.

31. Johnston CE RB. Other Anatomic Disorders of the Spine In: Saunders, ed. *Tachdjian's Pediatric Orthopaedics.* Vol 5 Elsevier 2014:328–355.

32. Ramo BA, Roberts DW, Tuason D, et al. Surgical site infections after posterior spinal fusion for neuromuscular scoliosis: a thirty-year experience at a single institution. *The Journal of bone and joint surgery American volume.* 2014;96(24):2038–2048. https://doi.org/10.2106/jbjs.n.00277.

33. Bradford DS, Iza J. Repair of the defect in spondylolysis or minimal degrees of spondylolisthesis by segmental wire fixation and bone grafting. *Spine (Phila Pa 1976).* 1985;10(7):673–679. https://doi.org/10.1097/00007632-198509000-00014.

34. Giudici F, Minoia L, Archetti M, Corriero AS, Zagra A. Long-term results of the direct repair of spondylolisthesis. *Eur Spine J.* 2011;20 Suppl 1(Suppl 1):S115–120. https://doi.org/10.1007/s00586-011-1759-9.

35. Johnson GV, Thompson AG. The Scott wiring technique for direct repair of lumbar spondylolysis. *J Bone Joint Surg Br.* 1992;74(3):426–430.

36. VanDam B. Nonoperative treatment and surgical repair of lumbar spondylolysis. In: Lipincott-Raven, ed. *The textbook of spinal surgery* Philadelphia, PA1997:1263.

37. Copley L. Disorders of the Neck. In: Saunders, ed. *Tachdijian's Pediatric Orthopaedics* Vol E4 Elsevier 2014:167–205.

38. Fielding JW, Hensinger RN, Hawkins RJ. Os Odontoideum. *J Bone Joint Surg Am.* 1980;62(3):376–383.

39. Wollin DG. THE OS ODONTOIDEUM. SEPARATE ODONTOID PROCESS. *J Bone Joint Surg Am.* 1963;45:1459–1471.

40. Gallie W. Fractures and Dislocations of Cervical Spine *Am J Surg.* 1939;46:495–499.

41. Loder RT. The Cervical Spine *Lovell and Winter's Pediatric Orthopaedics* Wolters Kluwer; 2012:821–885.

42. Ebersold MJ, Quast LM, Bianco AJ, Jr. Results of lumbar discectomy in the pediatric patient. *J Neurosurg.* 1987;67(5):643–647. https://doi.org/10.3171/jns.1987.67.5.0643.

43. Webb JH, Svien HJ, Kennedy RL. Protruded lumbar intervertebral disks in children. *J Am Med Assoc.* 1954;154(14):1153–1154. https://doi.org/10.1001/jama.1954.02940480005002.

44. Clarke NM, Cleak DK. Intervertebral lumbar disc prolapse in children and adolescents. *J Pediatr Orthop.* 1983;3(2):202–206. https://doi.org/10.1097/01241398-198305000-00009.

45. DeOrio JK, Bianco AJ, Jr. Lumbar disc excision in children and adolescents. *J Bone Joint Surg Am.* 1982;64(7):991–996.

46. Epstein JA, Epstein NE, Marc J, Rosenthal AD, Lavine LS. Lumbar intervertebral disk herniation in teenage children: recognition and management of associated anomalies. *Spine (Phila Pa 1976).* 1984;9(4):427–432. https://doi.org/10.1097/00007632-198405000-00019.

47. Papagelopoulos PJ, Shaughnessy WJ, Ebersold MJ, Bianco AJ, Jr., Quast LM. Long-term outcome of lumbar discectomy in children and adolescents sixteen years of age or younger. *J Bone Joint Surg Am.* 1998;80(5):689–698. https://doi.org/10.2106/00004623-199805000-00009.

48. Glotzbecker MP, Riedel MD, Vitale MG, et al. What's the evidence? Systematic literature review of risk factors and preventive strategies for surgical site infection following pediatric spine surgery. *J Pediatr Orthop.* 2013;33(5):479–487. https://doi.org/10.1097/BPO.0b013e318285c507.

49. Mistovich RJ, Jacobs L, Campbell R, Spiegel D, Flynn J, Baldwin KD. Infection Control in Pediatric Spinal Deformity Surgery: A Critical Analysis of Cause and Prevention Strategies in Adolescent Idiopathic Scoliosis, Neuromuscular Scoliosis, and Early Onset Scoliosis. *Pediatrics.* 2018;142(1 MeetingAbstract):328. https://doi.org/10.1542/peds.142.1_MeetingAbstract.328-a.

50. Subramanyam R, Schaffzin J, Cudilo EM, Rao MB, Varughese AM. Systematic review of risk factors for surgical site infection in pediatric scoliosis surgery. *Spine J.* 2015;15(6):1422–1431. https://doi.org/10.1016/j.spinee.2015.03.005.

51. Perry JW, Montgomerie JZ, Swank S, Gilmore DS, Maeder K. Wound infections following spinal fusion with posterior segmental spinal instrumentation. *Clin Infect Dis.* 1997;24(4):558–561. https://doi.org/10.1093/clind/24.4.558.

52. Brook I, Frazier EH. Aerobic and anaerobic microbiology of wound infection following spinal fusion in children. *Pediatr Neurosurg.* 2000;32(1):20–23. https://doi.org/10.1159/000028892.

53. Anari JB, Spiegel DA, Baldwin KD. Neuromuscular scoliosis and pelvic fixation in 2015: Where do we stand? *World J Orthop.* 2015;6(8):564–566. https://doi.org/10.5312/wjo.v6.i8.564.

54. Basques BA, Chung SH, Lukasiewicz AM, et al. Predicting Short-term Morbidity in Patients Undergoing Posterior Spinal Fusion for Neuromuscular Scoliosis. *Spine (Phila Pa 1976).* 2015;40(24):1910–1917. https://doi.org/10.1097/brs.0000000000001093.

55. Martin CT, Pugely AJ, Gao Y, Ilgenfritz RM, Weinstein SL. Incidence and risk factors for early wound complications after spinal arthrodesis in children: analysis of 30-day follow-up data from the ACS-NSQIP. *Spine (Phila Pa 1976).* 2014;39(18):1463–1470. https://doi.org/10.1097/brs.0000000000000446.

56. Mackenzie WG, Matsumoto H, Williams BA, et al. Surgical site infection following spinal instrumentation for scoliosis: a multicenter analysis of rates, risk factors, and pathogens. *J Bone Joint Surg Am.* 2013;95(9):800–806, s801–802. https://doi.org/10.2106/jbjs.L.00010.

57. Aleissa S, Parsons D, Grant J, Harder J, Howard J. Deep wound infection following pediatric scoliosis surgery: incidence and analysis of risk factors. *Can J Surg.* 2011;54(4):263–269. https://doi.org/10.1503/cjs.008210.

58. Barriga A, Díaz-de-Rada P, Barroso JL, et al. Frozen cancellous bone allografts: positive cultures of implanted grafts in posterior fusions of the spine. *Eur Spine J.* 2004;13(2):152–156. https://doi.org/10.1007/s00586-003-0633-9.

59. Lee FH, Shen PC, Jou IM, Li CY, Hsieh JL. A Population-Based 16-Year Study on the Risk Factors of Surgical Site Infection in Patients after Bone Grafting: A Cross-Sectional Study in Taiwan. *Medicine (Baltimore).* 2015;94(47):e2034. https://doi.org/10.1097/md.0000000000002034.

60. Ransford AO, Morley T, Edgar MA, et al. Synthetic porous ceramic compared with autograft in scoliosis surgery. A prospective, randomized study of 341 patients. *J Bone Joint Surg Br.* 1998;80(1):13–18. https://doi.org/10.1302/0301-620x.80b1.7276.

61. Soultanis KC, Pyrovolou N, Zahos KA, et al. Late postoperative infection following spinal instrumentation: stainless steel versus titanium implants. *J Surg Orthop Adv.* 2008;17(3):193–199.

62. Di Silvestre M, Bakaloudis G, Lolli F, Giacomini S. Late-developing infection following posterior fusion for adolescent idiopathic scoliosis. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European*

Section of the Cervical Spine Research Society. 2011;20 Suppl 1(Suppl 1):S121–S127. https://doi.org/10.1007/s00586-011-1754-1.

63. Glotzbecker MP, Gomez JA, Miller PE, et al. Management of Spinal Implants in Acute Pediatric Surgical Site Infections: A Multicenter Study. *Spine Deform.* 2016;4(4):277–282. https://doi.org/10.1016/j.jspd.2016.02.001.

64. Sheehan E, McKenna J, Mulhall KJ, Marks P, McCormack D. Adhesion of Staphylococcus to orthopaedic metals, an in vivo study. *J Orthop Res.* 2004;22(1):39–43. https://doi.org/10.1016/s0736-0266(03)00152-9.

65. Simmons EH, Bhalla SK. Anterior cervical discectomy and fusion. A clinical and biomechanical study with eight-year follow-up. *J Bone Joint Surg Br.* 1969;51(2):225–237.

66. Smith GW, Robinson RA. The treatment of certain cervical-spine disorders by anterior removal of the intervertebral disc and interbody fusion. *J Bone Joint Surg Am.* 1958;40–a(3):607–624.

67. Muschik M, Lück W, Schlenzka D. Implant removal for late-developing infection after instrumented posterior spinal fusion for scoliosis: reinstrumentation reduces loss of correction. A retrospective analysis of 45 cases. *Eur Spine J.* 2004;13(7):645–651. https://doi.org/10.1007/s00586-004-0694-4.

68. Clark CE, Shufflebarger HL. Late-developing infection in instrumented idiopathic scoliosis. *Spine (Phila Pa 1976).* 1999;24(18):1909–1912. https://doi.org/10.1097/00007632-199909150-00008.

69. Kowalski TJ, Berbari EF, Huddleston PM, Steckelberg JM, Mandrekar JN, Osmon DR. The management and outcome of spinal implant infections: contemporary retrospective cohort study. *Clin Infect Dis.* 2007;44(7):913–920. https://doi.org/10.1086/512194.

70. Ramage G, Tunney MM, Patrick S, Gorman SP, Nixon JR. Formation of Propionibacterium acnes biofilms on orthopaedic biomaterials and their susceptibility to antimicrobials. *Biomaterials.* 2003;24(19):3221–3227. https://doi.org/10.1016/s0142-9612(03)00173-x.

71. Ha KY, Chung YG, Ryoo SJ. Adherence and biofilm formation of Staphylococcus epidermidis and Mycobacterium tuberculosis on various spinal implants. *Spine (Phila Pa 1976).* 2005;30(1):38–43. https://doi.org/10.1097/01.brs.0000147801.63304.8a.

72. Maesani M, Doit C, Lorrot M, et al. Surgical Site Infections in Pediatric Spine Surgery: Comparative Microbiology of Patients with Idiopathic and Nonidiopathic Etiologies of Spine Deformity. *Pediatr Infect Dis J.* 2016;35(1):66–70. https://doi.org/10.1097/inf.0000000000000925.

73. Master DL, Poe-Kochert C, Son-Hing J, Armstrong DG, Thompson GH. Wound infections after surgery for neuromuscular scoliosis: risk factors and treatment outcomes. *Spine (Phila Pa 1976).* 2011;36(3):E179–185. https://doi.org/10.1097/BRS.0b013e3181db7afe.

74. Messina AF, Berman DM, Ghazarian SR, et al. The management and outcome of spinal implant-related infections in pediatric patients: a retrospective review. *Pediatr Infect Dis J.* 2014;33(7):720–723. https://doi.org/10.1097/inf.0000000000000264.

75. Glotzbecker MP, Vitale MG, Shea KG, Flynn JM. Surgeon practices regarding infection prevention for pediatric spinal surgery. *J Pediatr Orthop.* 2013;33(7):694–699. https://doi.org/10.1097/BPO.0b013e31829241b8.

76. Hahn F, Zbinden R, Min K. Late implant infections caused by Propionibacterium acnes in scoliosis surgery. *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society.* 2005;14(8):783–788. https://doi.org/10.1007/s00586-004-0854-6.

77. LaGreca J, Hotchkiss M, Carry P, et al. Bacteriology and Risk Factors for Development of Late (Greater Than One Year) Deep Infection Following Spinal Fusion With Instrumentation. *Spine Deform.* 2014;2(3):186–190. https://doi.org/10.1016/j.jspd.2013.12.004.

78. Lamberet A, Violas P, Buffet-Bataillon S, et al. Postoperative Spinal Implant Infections in Children: Risk Factors, Characteristics and Outcome. *Pediatr Infect Dis J.* 2018;37(6):511–513. https://doi.org/10.1097/inf.0000000000001812.

79. Minkara AA, Matsumoto H, Glotzbecker M, et al. A Multicenter Study of the Epidemiology of Deep Surgical Site Infections in Children With Nonidiopathic Early-Onset Scoliosis

Including Associated Pathogens. *Spine Deform.* 2019;7(4):647–651. https://doi.org/10.1016/j.jspd.2018.11.015.

80. Soultanis K, Mantelos G, Pagiatakis A, Soucacos PN. Late infection in patients with scoliosis treated with spinal instrumentation. *Clin Orthop Relat Res.* 2003(411):116–123. https://doi.org/10.1097/01.blo.0000068357.47147.10.

81. Richards BR, Emara KM. Delayed infections after posterior TSRH spinal instrumentation for idiopathic scoliosis: revisited. *Spine (Phila Pa 1976).* 2001;26(18):1990–1996. https://doi.org/10.1097/00007632-200109150-00009.

82. Murphy EC, Frick IM. Gram-positive anaerobic cocci--commensals and opportunistic pathogens. *FEMS Microbiol Rev.* 2013;37(4):520–553. https://doi.org/10.1111/1574-6976.12005.

83. Meredith DS, Kepler CK, Huang RC, Brause BD, Boachie-Adjei O. Postoperative infections of the lumbar spine: presentation and management. *Int Orthop.* 2012;36(2):439–444. https://doi.org/10.1007/s00264-011-1427-z.

84. Wagner L, Braunschweig L, Eiffert H, et al. Detection of Bacteria Colonizing Titanium Spinal Implants in Children. *Surg Infect (Larchmt).* 2018;19(1):71–77. https://doi.org/10.1089/sur.2017.185.

85. Bereza P, Ekiel A, Auguściak-Duma A, et al. Comparison of cultures and 16S rRNA sequencing for identification of bacteria in two-stage revision arthroplasties: preliminary report. *BMC Musculoskelet Disord.* 2016;17:138. https://doi.org/10.1186/s12891-016-0991-1.

86. Savage JW, Weatherford BM, Sugrue PA, et al. Efficacy of surgical preparation solutions in lumbar spine surgery. *J Bone Joint Surg Am.* 2012;94(6):490–494. https://doi.org/10.2106/jbjs.K.00471.

87. Darouiche RO, Wall MJ, Itani KMF, et al. Chlorhexidine–Alcohol versus Povidone–Iodine for Surgical-Site Antisepsis. *N Engl J Med.* 2010;362(1):18–26. https://doi.org/10.1056/NEJMoa0810988. Accessed 2020/07/01.

88. Boston KM, Baraniuk S, O'Heron S, Murray KO. Risk factors for spinal surgical site infection, Houston, Texas. *Infect Control Hosp Epidemiol.* 2009;30(9):884–889. https://doi.org/10.1086/605323.

89. Vitale MG, Riedel MD, Glotzbecker MP, et al. Building consensus: development of a Best Practice Guideline (BPG) for surgical site infection (SSI) prevention in high-risk pediatric spine surgery. *J Pediatr Orthop.* 2013;33(5):471–478. https://doi.org/10.1097/BPO.0b013e3182840de2.

90. Hatlen T, Song K, Shurtleff D, Duguay S. Contributory factors to postoperative spinal fusion complications for children with myelomeningocele. *Spine (Phila Pa 1976).* 2010;35(13):1294–1299. https://doi.org/10.1097/brs.0b013e3181bf8efe.

91. Verhoef M, Lurvink M, Barf HA, et al. High prevalence of incontinence among young adults with spina bifida: description, prediction and problem perception. *Spinal Cord.* 2005;43(6):331–340. https://doi.org/10.1038/sj.sc.3101705.

92. Núñez-Pereira S, Pellisé F, Rodríguez-Pardo D, et al. Individualized antibiotic prophylaxis reduces surgical site infections by gram-negative bacteria in instrumented spinal surgery. *Eur Spine J.* 2011;20 Suppl 3(Suppl 3):397–402. 10.1007/s00586-011-1906-3.

93. Watters WC, 3rd, Baisden J, Bono CM, et al. Antibiotic prophylaxis in spine surgery: an evidence-based clinical guideline for the use of prophylactic antibiotics in spine surgery. *Spine J.* 2009;9(2):142–146. https://doi.org/10.1016/j.spinee.2008.05.008.

94. Luhmann SJ, Smith JC. Preoperative MRSA Screening in Pediatric Spine Surgery: A Helpful Tool or a Waste of Time and Money? *Spine deformity.* 2016;4(4):272–276. https://doi.org/10.1016/j.jspd.2015.12.006.

95. Andersson, A.E.; Bergh, I.; Karlsson, J.; Eriksson, B.I.; Nilsson, K. Traffic flow in the operating room: an explorative and descriptive study on air quality during orthopedic trauma implant surgery. Am. J. Infect. Control., 2012, 40, 750–5.

96. Borst, M.; Collier, C.; Miller, D. Operating room surveillance: a new approach in reducing hip and knee prosthetic wound infections. Am. J. Infect. Control., 1986, 14, 161–6

97. Pokrywka M, Byers K. Traffic in the operating room: a review of factors influencing air flow and surgical wound contamination. *Infect Disord Drug Targets*. 2013;13(3):156–161. doi:https://doi.org/10.2174/1871526511313030002

98. Savolaine ER, Ebraheim NA, Andreshak TG, Jackson WT. Anterior and posterior cervical spine fixation using titanium implants to facilitate magnetic resonance imaging evaluation. *J Orthop Trauma.* 1989;3(4):295–299. https://doi.org/10.1097/00005131-198912000-00006.

99. Cheng M-T, Chang M-C, Wang S-T, Yu W-K, Liu C-L, Chen T-H. Efficacy of dilute betadine solution irrigation in the prevention of postoperative infection of spinal surgery. *Spine (Phila Pa 1976).* 2005;30(15):1689–1693. https://doi.org/10.1097/01.brs.0000171907.60775.85.

100. Chang FY, Chang MC, Wang ST, Yu WK, Liu CL, Chen TH. Can povidone-iodine solution be used safely in a spinal surgery? *Eur Spine J.* 2006;15(6):1005–1014. https://doi.org/10.1007/s00586-005-0975-6.

101. Newton Ede MP, Philp AM, Philp A, Richardson SM, Mohammad S, Jones SW. Povidone-Iodine Has a Profound Effect on In Vitro Osteoblast Proliferation and Metabolic Function and Inhibits Their Ability to Mineralize and Form Bone. *Spine (Phila Pa 1976).* 2016;41(9):729–734. https://doi.org/10.1097/brs.0000000000001332.

102. Ryan M, Al-Sammak Z, Phelan D. Povidone-iodine mediastinal irrigation: a cause of acute renal failure. *J Cardiothorac Vasc Anesth.* 1999;13(6):729–731. https://doi.org/10.1016/s1053-0770(99)90130-1.

103. Bridwell KH, O'Brien MF, Lenke LG, Baldus C, Blanke K. Posterior spinal fusion supplemented with only allograft bone in paralytic scoliosis. Does it work? *Spine (Phila Pa 1976).* 1994;19(23):2658–2666.

104. McCarthy RE, Peek RD, Morrissy RT, Hough AJ, Jr. Allograft bone in spinal fusion for paralytic scoliosis. *JBJS.* 1986;68(3). https://journals.lww.com/jbjsjournal/Fulltext/1986/68030/Allograft_bone_in_spinal_fusion_for_paralytic.9.aspx.

105. Grogan DP, Kalen V, Ross TI, Guidera KJ, Pugh LI. Use of allograft bone for posterior spinal fusion in idiopathic scoliosis. *Clin Orthop Relat Res.* 1999(369):273–278. https://doi.org/10.1097/00003086-199912000-00028.

106. Jones KC, Andrish J, Kuivila T, Gurd A. Radiographic outcomes using freeze-dried cancellous allograft bone for posterior spinal fusion in pediatric idiopathic scoliosis. *J Pediatr Orthop.* 2002;22(3):285–289.

107. Mohamed Ali MH, Koutharawu DN, Miller F, et al. Operative and clinical markers of deep wound infection after spine fusion in children with cerebral palsy. *J Pediatr Orthop.* 2010;30(8):851–857. https://doi.org/10.1097/BPO.0b013e3181f59f3f.

108. Sweet FA, Roh M, Sliva C. Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. *Spine (Phila Pa 1976).* 2011;36(24):2084–2088. https://doi.org/10.1097/BRS.0b013e3181ff2cb1.

109. Khan NR, Thompson CJ, DeCuypere M, et al. A meta-analysis of spinal surgical site infection and vancomycin powder. *J Neurosurg Spine.* 2014;21(6):974–983. https://doi.org/10.3171/2014.8.Spine1445.

110. Haller JM, Heflin JA, Hulet DA, Ding Q, Presson AP, Smith JT. Intrawound Vancomycin Powder Associated With Reduced Surgical Site Infection in Rib-based Distraction Surgery. *Journal of Pediatric Orthopaedics.* 2019;39(9). https://journals.lww.com/pedorthopaedics/Fulltext/2019/10000/Intrawound_Vancomycin_Powder_Associated_With.12.aspx.

111. Soriano A, Marco F, Martínez JA, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant Staphylococcus aureus bacteremia. *Clin Infect Dis.* 2008;46(2):193–200. https://doi.org/10.1086/524667.

112. Neoh HM, Hori S, Komatsu M, et al. Impact of reduced vancomycin susceptibility on the therapeutic outcome of MRSA bloodstream infections. *Ann Clin Microbiol Antimicrob.* 2007;6:13. https://doi.org/10.1186/1476-0711-6-13.

113. Gans I, Dormans JP, Spiegel DA, et al. Adjunctive vancomycin powder in pediatric spine surgery is safe. *Spine (Phila Pa 1976).* 2013;38(19):1703–1707. https://doi.org/10.1097/BRS.0b013e31829e05d3.

114. Philp AM, Raja S, Philp A, Newton Ede MP, Jones SW. The Effect of Vancomycin and Gentamicin Antibiotics on Human Osteoblast Proliferation, Metabolic Function, and Bone Mineralization. *Spine (Phila Pa 1976).* 2017;42(3):202–207. https://doi.org/10.1097/brs.0000000000001712.

115. Tubaki VR, Rajasekaran S, Shetty AP. Effects of using intravenous antibiotic only versus local intrawound vancomycin antibiotic powder application in addition to intravenous antibiotics on postoperative infection in spine surgery in 907 patients. *Spine (Phila Pa 1976).* 2013;38(25):2149–2155. https://doi.org/10.1097/brs.0000000000000015.

116. Xu L, Zhu F, Zhu Z, et al. Comparison of 2 methods of incision closure in patients with adolescent idiopathic scoliosis undergoing posterior spinal fusion surgery. *Spine (Phila Pa 1976).* 2014;39(8):E481–485. https://doi.org/10.1097/brs.0000000000000223.

117. Mansour A, Ballard R, Garg S, Baulesh D, Erickson M. The use of barbed sutures during scoliosis fusion wound closure: a quality improvement analysis. *J Pediatr Orthop.* 2013;33(8):786–790. https://doi.org/10.1097/BPO.0b013e3182a11eee.

118. Dromzee E, Tribot-Laspière Q, Bachy M, Zakine S, Mary P, Vialle R. Efficacy of integuseal for surgical skin preparation in children and adolescents undergoing scoliosis correction. *Spine (Phila Pa 1976).* 2012;37(21):E1331–1335. https://doi.org/10.1097/BRS.0b013e3182687d6c.

119. Eickmann T, Quane E. Total knee arthroplasty closure with barbed sutures. *J Knee Surg.* 2010;23(3):163–167. https://doi.org/10.1055/s-0030-1268692.

120. Stephens S, Politi J, Taylor BC. Evaluation of Primary Total Knee Arthroplasty Incision Closure with the Use of Continuous Bidirectional Barbed Suture. *Surg Technol Int.* 2011;21:199–203.

121. Ting NT, Moric MM, Della Valle CJ, Levine BR. Use of knotless suture for closure of total hip and knee arthroplasties: a prospective, randomized clinical trial. *J Arthroplasty.* 2012;27(10):1783–1788. https://doi.org/10.1016/j.arth.2012.05.022.

122. Takahashi H, Wada A, Iida Y, et al. Antimicrobial prophylaxis for spinal surgery. *J Orthop Sci.* 2009;14(1):40–44. https://doi.org/10.1007/s00776-008-1296-5.

123. Rimoldi RL, Haye W. The use of antibiotics for wound prophylaxis in spinal surgery. *Orthop Clin North Am.* 1996;27(1):47–52.

124. Marimuthu C, Abraham VT, Ravichandran M, Achimuthu R. Antimicrobial Prophylaxis in Instrumented Spinal Fusion Surgery: A Comparative Analysis of 24-Hour and 72-Hour Dosages. *Asian Spine J.* 2016;10(6):1018–1022. https://doi.org/10.4184/asj.2016.10.6.1018.

125. Kamath VH, Cheung JP, Mak KC, et al. Antimicrobial prophylaxis to prevent surgical site infection in adolescent idiopathic scoliosis patients undergoing posterior spinal fusion: 2 doses versus antibiotics till drain removal. *Eur Spine J.* 2016;25(10):3242–3248. https://doi.org/10.1007/s00586-016-4491-7.

126. Bains RS, Kardile M, Mitsunaga LK, Bains S, Singh N, Idler C. Postoperative Spine Dressing Changes Are Unnecessary. *Spine Deformity.* 2017;5(6):396–400. https://doi.org/10.1016/j.jspd.2017.04.005.

127. Narayan P BM, Gould J. Use of silver-impregnated dressings to reduce neurosurgical infections. 42nd Annual Meeting of the International Society for Pediatric Neurosurgery Nov 9–13, 2014; Rio De Janeiro, Brazil

128. Holt BT, Parks NL, Engh GA, Lawrence JM. Comparison of closed-suction drainage and no drainage after primary total knee arthroplasty. *Orthopedics.* 1997;20(12):1121–1124; discussion 1124–1125.

129. Kim YH, Cho SH, Kim RS. Drainage versus nondrainage in simultaneous bilateral total knee arthroplasties. *Clin Orthop Relat Res.* 1998;(347):188–193.

130. Kochai A, Erkorkmaz Ü. The role of drains in adolescent idiopathic scoliosis surgery: Is it necessary? *Medicine.* 2019;98(51):e18061–e18061. https://doi.org/10.1097/MD.0000000000018061.

131. Parker MJ, Livingstone V, Clifton R, McKee A. Closed suction surgical wound drainage after orthopaedic surgery. *Cochrane Database Syst Rev.* 2007(3):Cd001825. https://doi.org/10.1002/14651858.CD001825.pub2.

132. Diab M, Smucny M, Dormans JP, et al. Use and outcomes of wound drain in spinal fusion for adolescent idiopathic scoliosis. *Spine (Phila Pa 1976).* 2012;37(11):966–973. https://doi.org/10.1097/BRS.0b013e31823bbf0b.

133. Fichman SG, Mäkinen TJ, Lozano B, et al. Closed suction drainage has no benefits in revision total hip arthroplasty: a randomized controlled trial. *Int Orthop.* 2016;40(3):453–457. doi: https://doi.org/10.1007/s00264-015-2960-y.

134. Zhang Q, Zhang Q, Guo W, Liu Z, Cheng L, Zhu G. No need for use of drainage after minimally invasive unicompartmental knee arthroplasty: a prospective randomized, controlled trial. *Arch Orthop Trauma Surg.* 2015;135(5):709–713. https://doi.org/10.1007/s00402-015-2192-z.

135. Blank J, Flynn JM, Bronson W, et al. The use of postoperative subcutaneous closed suction drainage after posterior spinal fusion in adolescents with idiopathic scoliosis. *J Spinal Disord Tech.* 2003;16(6):508–512. https://doi.org/10.1097/00024720-200312000-00004.

136. Brown MD, Brookfield KF. A randomized study of closed wound suction drainage for extensive lumbar spine surgery. *Spine (Phila Pa 1976).* 2004;29(10):1066–1068. https://doi.org/10.1097/00007632-200405150-00003.

137. Liu Y, Li Y, Miao J. Wound drains in posterior spinal surgery: a meta-analysis. *J Orthop Surg Res.* 2016;11:16. https://doi.org/10.1186/s13018-016-0351-8.

138. Croft LD, Pottinger JM, Chiang HY, Ziebold CS, Weinstein SL, Herwaldt LA. Risk factors for surgical site infections after pediatric spine operations. *Spine (Phila Pa 1976).* 2015;40(2):E112–119. https://doi.org/10.1097/brs.0000000000000693.

139. Alsiddiky A, Nisar KA, Alhuzaimi F, et al. Wound healing without drains in posterior spinal fusion in idiopathic scoliosis. *J Coll Physicians Surg Pak.* 2013;23(8):558–561.

140. Kleinert K, Werner C, Mamisch-Saupe N, Kalberer F, Dora C. Closed suction drainage with or without re-transfusion of filtered shed blood does not offer advantages in primary non-cemented total hip replacement using a direct anterior approach. *Arch Orthop Trauma Surg.* 2012;132(1):131–136. https://doi.org/10.1007/s00402-011-1387-1.

141. Lwin S, Low SW, Choy DK, Yeo TT, Chou N. External ventricular drain infections: successful implementation of strategies to reduce infection rate. *Singapore Med J.* 2012;53(4):255–259.

142. van Rhee MA, de Klerk LW, Verhaar JA. Vacuum-assisted wound closure of deep infections after instrumented spinal fusion in six children with neuromuscular scoliosis. *Spine J.* 2007;7(5):596–600. https://doi.org/10.1016/j.spinee.2006.09.002.

143. Canavese F, Gupta S, Krajbich JI, Emara KM. Vacuum-assisted closure for deep infection after spinal instrumentation for scoliosis. *J Bone Joint Surg Br.* 2008;90(3):377–381. https://doi.org/10.1302/0301-620x.90b3.19890.

144. Yuan-Innes MJ, Temple CL, Lacey MS. Vacuum-assisted wound closure: a new approach to spinal wounds with exposed hardware. *Spine (Phila Pa 1976).* 2001;26(3):E30–33. https://doi.org/10.1097/00007632-200102010-00006.

145. Mehbod AA, Ogilvie JW, Pinto MR, et al. Postoperative deep wound infections in adults after spinal fusion: management with vacuum-assisted wound closure. *J Spinal Disord Tech.* 2005;18(1):14–17. https://doi.org/10.1097/01.bsd.0000133493.32503.d3.

146. Bihariesingh VJ, Stolarczyk EM, Karim RB, van Kooten EO. Plastic solutions for orthopaedic problems. *Arch Orthop Trauma Surg.* 2004;124(2):73–76. https://doi.org/10.1007/s00402-003-0615-8.

147. Argenta LC, Morykwas MJ. Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. *Ann Plast Surg.* 1997;38(6):563–576; discussion 577.

148. Early SD, Kay RM, Tolo VT. Childhood diskitis. *J Am Acad Orthop Surg.* 2003;11(6):413–420. https://doi.org/10.5435/00124635-200311000-00005.

149. Fernandez M, Carrol CL, Baker CJ. Discitis and vertebral osteomyelitis in children: an 18-year review. *Pediatrics.* 2000;105(6):1299–1304. https://doi.org/10.1542/peds.105.6.1299.

150. Silber JS, Anderson DG, Vaccaro AR, Anderson PA, McCormick P. Management of postprocedural discitis. *Spine J.* 2002;2(4):279–287. https://doi.org/10.1016/s1529-9430(02)00203-6.

151. Osmon DR, Berbari EF, Berendt AR, et al. Diagnosis and Management of Prosthetic Joint Infection: Clinical Practice Guidelines by the Infectious Diseases Society of America. *Clin Infect Dis.* 2012;56(1):e1–e25. https://doi.org/10.1093/cid/cis803. Accessed 7/1/2020.

152. Lora-Tamayo J, Senneville É, Ribera A, et al. The Not-So-Good Prognosis of Streptococcal Periprosthetic Joint Infection Managed by Implant Retention: The Results of a Large Multicenter Study. *Clin Infect Dis.* 2017;64(12):1742–1752. https://doi.org/10.1093/cid/cix227.

153. Martínez-Pastor JC, Muñoz-Mahamud E, Vilchez F, et al. Outcome of acute prosthetic joint infections due to gram-negative bacilli treated with open debridement and retention of the prosthesis. *Antimicrob Agents Chemother.* 2009;53(11):4772–4777. https://doi.org/10.1128/aac.00188-09.

154. Rodríguez-Pardo D, Pigrau C, Lora-Tamayo J, et al. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. *Clin Microbiol Infect.* 2014;20(11):O911–919. https://doi.org/10.1111/1469-0691.12649.

155. Bradley JS, Jackson MA; Committee on Infectious Diseases; American Academy of Pediatrics. The use of systemic and topical fluoroquinolones. Pediatrics. 2011 Oct;128(4):e1034–45. https://doi.org/10.1542/peds.2011-1496. Epub 2011 Sep 26.

156. Gepstein R EF. Postoperative spine infections. In: SR G, ed. *Complications of Spine Surgery* Baltimore, MD: Wiliams and Wilkins 1989:302–322.

157. Mitra A, Mitra A, Harlin S. Treatment of massive thoracolumbar wounds and vertebral osteomyelitis following scoliosis surgery. *Plast Reconstr Surg.* 2004;113(1):206–213. https://doi.org/10.1097/01.Prs.0000097440.15013.5c.

158. Collins I, Wilson-MacDonald J, Chami G, et al. The diagnosis and management of infection following instrumented spinal fusion. *Eur Spine J.* 2008;17(3):445–450. https://doi.org/10.1007/s00586-007-0559-8.

159. Skaggs D HC, Weiss J, Tolo V. Management of Infection in pediatric scoliosis fusions. 40th Annual Meeting of the Scoliosis Research Society 2005; Miami, FL

160. Rathjen K, Wood M, McClung A, Vest Z. Clinical and radiographic results after implant removal in idiopathic scoliosis. *Spine (Phila Pa 1976).* 2007;32(20):2184–2188. https://doi.org/10.1097/BRS.0b013e31814b88a5.

161. Khoshbin A, Lysenko M, Law P, Wright JG. Outcomes of infection following pediatric spinal fusion. *Can J Surg.* 2015;58(2):107–113. https://doi.org/10.1503/cjs.006014.

162. Viola RW, King HA, Adler SM, Wilson CB. Delayed infection after elective spinal instrumentation and fusion. A retrospective analysis of eight cases. *Spine (Phila Pa 1976).* 1997;22(20):2444–2450; discussion 2450–2441. https://doi.org/10.1097/00007632-199710150-00023.

163. Richards BS. Delayed infections following posterior spinal instrumentation for the treatment of idiopathic scoliosis. *J Bone Joint Surg Am.* 1995;77(4):524–529. https://doi.org/10.2106/00004623-199504000-00004.

164. Gómez Cáceres A, Lucena Jiménez JS, Reyes Martín Á L, Moriel Durán J, Sobrino Diaz B, García de Quevedo Puerta D. Prognosis of deep infection in spinal surgery using implants, treated by retention, removal of bone graft and lengthy antibiotherapy. *Rev Esp Cir Ortop Traumatol.* 2019;63(1):7–11. https://doi.org/10.1016/j.recot.2018.10.001.

165. Inose H, Kobayashi Y, Yuasa M, Hirai T, Yoshii T, Okawa A. Procalcitonin and Neutrophil Lymphocyte Ratio After Spinal Instrumentation Surgery. *Spine (Phila Pa 1976).* 2019;44(23):E1356–e1361. https://doi.org/10.1097/brs.0000000000003157.

166. Inose H, Kobayashi Y, Yuasa M, Hirai T, Yoshii T, Okawa A. Postoperative lymphocyte percentage and neutrophil-lymphocyte ratio are useful markers for the early prediction of surgical site infection in spinal decompression surgery. *J Orthop Surg (Hong Kong).* 2020;28(2):2309499020918402. https://doi.org/10.1177/2309499020918402.

167. Shen CJ, Miao T, Wang ZF, et al. Predictive value of post-operative neutrophil/lymphocyte count ratio for surgical site infection in patients following posterior lumbar spinal surgery. *Int Immunopharmacol.* 2019;74:105705. https://doi.org/10.1016/j.intimp.2019.105705.
168. Iwata E, Shigematsu H, Koizumi M, et al. Lymphocyte Count at 4 Days Postoperatively and CRP Level at 7 Days Postoperatively: Reliable and Useful Markers for Surgical Site Infection Following Instrumented Spinal Fusion. *Spine (Phila Pa 1976).* 2016;41(14):1173–1178. https://doi.org/10.1097/brs.0000000000001501.
169. Laratta JL, Lombardi JM, Shillingford JN, Reddy HP, Gvozdyev BV, Kim YJ. Permanent implantation of antibiotic cement over exposed instrumentation eradicates deep spinal infection. *J Spine Surg.* 2018;4(2):471–477. https://doi.org/10.21037/jss.2018.04.03.
170. Cancienne JM, Burrus MT, Weiss DB, Yarboro SR. Applications of Local Antibiotics in Orthopedic Trauma. *Orthop Clin North Am.* 2015;46(4):495–510. https://doi.org/10.1016/j.ocl.2015.06.010.

Chapter 11
War Wounds and Orthopedic Trauma Devices

Maj Dana M. Blyth and Col Heather C. Yun

Abstract The combined wars in Iraq and Afghanistan represent the longest ongoing conflicts in American military history, with current statistics showing more than 59,000 casualties wounded in action. Ultimately, combat-related extremity injuries require the longest inpatient stay (10.7 days), are responsible for 64% of total inpatient resource utilization, and disable 64% of those injured. Many of these patients require multiple surgical procedures, putting those with severe injuries and prolonged hospitalizations at risk for nosocomial and delayed infections, which we have only begun to evaluate systematically in the more recent conflicts. This chapter presents techniques for the initial management of war wounds, war wound infection, combat-related osteomyelitis, orthopedic device-related infections, fungal wound infections, as well as contemporary research and innovative approaches that have potential to move the field forward.

Keywords War wound · Implants · Infection · Management · Surgery · Infection control · Epidemiology · Osteomyelitis · Fungal wounds · Combat-related

11.1 Introduction

The combined wars in Iraq and Afghanistan represent the longest ongoing conflicts in American military history, with current statistics showing more than 59,000 casualties wounded in action [1, 2]. With rapid medical evacuation in modern warfare and decreased battlefield fatalities, physicians are faced with the challenge of managing increasingly complex combat-related injuries [3–9]. Modern warfare techniques, particularly the rise of unconventional ambush attacks and improvised

M. D. M. Blyth (✉)
Infectious Disease Service, Walter Reed National Military Medical Center, Bethesda, MD, USA
e-mail: dana.m.blyth.mil@mail.mil

C. H. C. Yun
Brooke Army, Medical Center, San Antonio, TX, USA
e-mail: heather.c.yun.mil@mail.mil

explosive devices (IEDs), have been associated with new wound patterns (especially dismounted complex blast injury [DCBI]), complications, and microbiology of infections [3, 8, 10–12]. The first widespread use of individual body armor and Kevlar helmets occurred in Iraq and Afghanistan, which reduced overall thoracic injuries and impact of otherwise fatal wounds. Those who survive these previously fatal injuries may have traumatic amputations, extensive soft tissue loss, gross contamination of wounds, and bone injury and have received massive transfusions. Nearly 75% of combat injuries are now secondary to explosive mechanisms, and 77% have least one orthopedic injury with fractures representing 40% of all musculoskeletal injuries and 6% of amputations. Ultimately, combat-related extremity injuries require the longest inpatient stay (10.7 days), are responsible for 64% of total inpatient resource utilization, and disable 64% of those injured [2, 13]. Many of these patients require multiple surgical procedures, putting those with severe injuries and prolonged hospitalizations at risk for nosocomial and delayed infections, which we have only begun to evaluate systematically in the more recent conflicts [14–16].

The most common mechanisms of injury and many challenges differ between war and civilian trauma, making research to clarify unique risk factors, complications, and management strategies imperative. Initial surgical management is driven by the pattern and mechanisms of injury. In prior and current conflicts, extremities have been the most common site of combat-injury. Overall, lower-extremity injuries account for approximately half of extremity injuries sustained during combat, are more severe, and have higher infection rates [17–20]. Lessons from prior wars led to centralization of databases (like the Joint Theater Trauma Registry [JTTR] and subsequent Department of Defense Trauma Registry [DoDTR] and Armed Forces Medical Examiner System [AFMES]). This has resulted in more systematic data gathering, research, and generation of clinical practice guidelines during Operation Iraqi Freedom (OIF)/Operation Enduring Freedom (OEF)/Operation New Dawn (OND) [2, 3, 21–23]. Particularly cogent to this chapter is the Trauma Infectious Disease Outcomes Study (TIDOS), which prospectively enrolled personnel medically evacuated from OIF/OEF through Landstuhl Regional Medical Center (LRMC) in Germany from 2009 to 2014, collecting standardized infection data from point-of-injury through Veterans Administration (VA) care and allowing more in-depth evaluation of the risks and complications of infectious complications of combat-related trauma [14, 24].

A recent study reviewed the first 3 years of clinical data from TIDOS, including over 3000 wounded military personnel evacuated to LRMC, of which more than 1800 were subsequently transferred to TIDOS-participating hospitals in the continental United States (CONUS). Notably, more than 90% of the patients included were from OEF. Severe or life-threatening injuries (injury severity scores [ISS] > 15) were present in more than a quarter of evacuees, blast injuries accounted for almost 70% of injuries (with more than 10% traumatic amputations), and more than a third required intensive care unit (ICU) admission during aeromedical evacuation. Overall, approximately one-third of combat casualties medically evacuated from

OIF/OEF developed infectious complications prior to their discharge from definitive hospitalization in the United States. It remains challenging to interpret these data in historical context because prior data is largely confined to analysis of infections during initial hospitalizations (which were often prolonged in theater). Important to keep in context from the TIDOS data is that those patients transferred to a TIDOS-participating CONUS hospital were more severely injured than the overall combat-injured population. Identified risk factors for infection were amputation, blood transfusions in the first 24 h, LRMC ICU admission, severe or life-threatening ISS, and mechanical ventilation. Of those with infections, more than half were skin and skin structure infections (SSTIs) and osteomyelitis, 15% bloodstream infections, and 15% pneumonia [15].

While bacteria isolated from war wounds in large studies of OEF/OIF are similar to those of Vietnam, the increased threat of multidrug-resistant bacteria and challenges of infection control in the deployed environment and modern medical evacuation chain continue to test providers [3, 15, 25, 26]. In this chapter, infectious complications of war wounds, with a particular concentration on SSTIs and wound infections, osteomyelitis, and orthopedic device-related infections, and recent research into strategies to mitigate the infectious complications of war wounds will be covered.

11.2 Initial Management of War Wounds

11.2.1 Prehospital Management and Point-of-Injury Antibiotics

In comparison to civilian trauma, which is primarily blunt force, the majority of combat injuries are penetrating injuries due to high energy mechanisms (blast or high-velocity gunshot wounds) which are frequently complicated by early wound contamination with dirt, soil, and individual's own clothing, as well as potential for delays in evacuation due to conditions of war [27]. Prehospital management of wounds includes liberal use of tourniquets for compressible hemorrhage and initial irrigation. Well-applied tourniquets are maintained from injury to operating room, with prior studies showing that proper training on application of tourniquets is the most crucial component for effectiveness. Non-compressible hemorrhage (i.e., axillary, groin, extremely proximal thigh) is managed with field application of hemostatic dressings as reduction in fatal hemorrhage outweighs the associated risks of burns, neurologic injury, and additional tissue damage [28, 29].

A recent study of open type III tibia fractures from the civilian literature revealed that delay of antimicrobials even 1 h following injury resulted in increased risk of infection [30]. Because of the urgency of antimicrobial administration in the setting of combat-related open wounds, high rates of fractures with associated injuries, and potential for prolonged field care, recommendations for point-of-injury antibiotics

(which are especially emphasized in the setting of anticipated delayed medical evacuation to surgical care) have been recommended as part of the Tactical Combat Casualty Care (TCCC) Guidelines since 2003 [17, 31]. Moxifloxacin is recommended if the patient is able to tolerate oral medications and ertapenem if the patient is unable to tolerate PO, unconscious, or critically ill (Table 11.1) [32, 33]. A study evaluating the efficacy of point-of-injury antimicrobials within the 75th Ranger Regiment prehospital trauma registry revealed that of 405 casualties injured between 2003 and 2010, prehospital antimicrobials were only administered in 113 (27.9%). Though limited by sample size and unknown time from injury to surgical care, there did not appear to be an increase in infection or colonization with multidrug-resistant organisms (MDROs) or decrease in subsequent infectious complications in those with point-of-injury antimicrobials, though power was limited to detect these outcomes [34].

11.2.2 Initial Surgical Management

With increased utilization of IEDs by enemy forces and increased survivability on the battlefield due to improved personal protective equipment and hemorrhage control, physicians have been faced with increasingly complex and severe extremity battlefield trauma [35]. Basic war surgery principles of aggressive resuscitation, early and thorough debridement, short duration damage control surgery, and rapid evacuation were critical in reductions of died of wounds (DOW) rates to <7% for all admissions in recent conflicts [35]. Rapid surgical debridement has traditionally been felt to be critical for reducing infection risk despite inconsistent literature supporting this. Recent studies have shown no difference in infection rates with varying times to debridement (as long as within 24 h). Recommendations are currently for debridement to be performed as early as feasible [32, 36–40]. In general, all severe injuries (whether blast or high-velocity gunshot wound) require meticulous surgical debridement with the wound left open, early fracture stabilization, antimicrobials, and rapid evacuation to a higher level of care [35].

Due to a combination of factors including limited radiologic support, availability of instruments and implant selection, uncertainty of mass casualty incidents, and unconfirmed sterility in the combat environment, damage control orthopedics (the use of external fixation to provide temporary stabilization of extremity injuries until safe, definitive treatment) remains standard practice in theater [28, 32, 41]. This attempt to balance the decrease in septic complications with the increased risk of scarring and contractures drives modern combat injury wound care [19]. Because definitive fixation and closure is typically performed CONUS and the patient will often have been through multiple echelons of care before definitive fixation and closure, the surgeon performing damage control orthopedics is rarely the surgeon performing definitive treatment. It is therefore critical to preserve approaches and hardware placement possibilities for as many future options as possible [28]. Unfortunately, there is limited data to evaluate internal versus external fixation

Table 11.1 Post-combat injury antimicrobial agents and duration[a]

Injury	Preferred agent(s)	Alternate agent(s)	Duration
Point-of-injury antimicrobials for delayed evacuation to surgical care with open combat wounds			
Able to take PO medications	Moxifloxacin 400 mg PO x1 dose	Levofloxacin 500 mg PO x1 dose	Single-dose therapy
Unable to take PO, OR shock	Ertapenem 1 g IV or IM	Cefotetan 2 g IV or IM q12 h	Single-dose therapy
Extremity wounds (including skin, soft tissue, and bone)			
Skin, soft tissue, no open fractures	Cefazolin 2 g IV q6–8 h[b]	Clindamycin 300–450 mg PO TID or 600 mg iv q8 h	1–3 days
Skin, soft tissue, with open fractures	Cefazolin 2 g IV q6–8 h[b]	Clindamycin 600 mg iv q8 h	1–3 days
Thoracic and abdominal wounds			
Penetrating chest injury without esophageal disruption	Cefazolin 2 g IV q 6–8 h[b]	Clindamycin 300–450 mg PO TID or 600 mg IV q8 h	1 day
Penetrating chest injury with esophageal disruption	Cefazolin 2 g IV q 6–8 h[b] PLUS metronidazole 500 mg IV q8–12 h	Ertapenem 1 gm IV x1; OR moxifloxacin 400 mg IV x1	1 day after definitive washout
Penetrating abdominal injury with suspected/ known viscus injury and soilage	Cefazolin 2 g IV q 6–8 h[b] PLUS metronidazole 500 mg IV q8–12 h	Ertapenem 1 g IV x1; OR moxifloxacin 400 mg IV x1	1 day after definitive washout
Maxillofacial and neck wounds			
Open maxillofacial fractures or maxillofacial fractures with foreign body or fixation device	Cefazolin 2 g IV q6–8 h[b]	Clindamycin 600 mg IV q8 h	1 day
Central nervous system wounds			
Penetrating brain injury	Cefazolin 2 g IV q6–8 h[b]; consider adding metronidazole 500 mg IV q8–12 h if gross contamination with organic debris	Ceftriaxone 2 g IV q24 h; consider adding metronidazole 500 mg IV q8–12 h if gross contamination with organic debris	5 days or until CSF leak is closed, whichever is longer
		For penicillin allergic: vancomycin 1 g IV q12 h PLUS ciprofloxacin 400 mg IV q8–12 h; consider addition of metronidazole as above	

Table 11.1 (continued)

Injury	Preferred agent(s)	Alternate agent(s)	Duration
Penetrating spinal cord injury	Cefazolin 2 g IV q6–8 h[b]; consider adding metronidazole 500 mg IV q8–12 h if abdominal cavity involved	As above, add metronidazole 500 mg IV q8–12 h if abdominal cavity involved	5 days or until CSF leak is closed, whichever is longer

Adapted from Murray CK, et al. Prevention of Infections Associated with Combat-Related Extremity Injuries. J Trauma 2011;71(No 2, Suppl 2):S235–257. Saeed O, et al. Joint Trauma System Clinical Practice Guideline, Infection Prevention in Combat-Related Injuries. (https://jts.amedd.army.mil/assets/docs/cpgs/JTS_Clinical_Practice_Guidelines_(CPGs)/Infection_Prevention_08_Aug_2016_ID24.pdf)

[a]In the event of blood loss of more than 1500 to 2000 mL, repeat perioperative antibiotic dosing is recommended within the 2- to 4-h period

[b]Cefazolin is dosed by body mass: weight < 80 kg (1 g), weight 81–160 kg (2 g), weight > 160 kg (3 g)

versus plaster casting (and avoidance of pin tracts) with subsequent development of infectious complications in these complicated and contaminated war wounds. Ultimately, the quality of the initial surgery, rather than the type of fixation, is likely the most important factor in determining the outcome [42]. Current US guidelines recommend external fixation for femur and tibia fractures in combat-theater hospitals [28, 41]. Conversion to internal fixation during CONUS hospitalization following appropriate wound management is then recommended. Conversion earlier, at LRMC, Germany, during evacuation remains controversial [41].

There have been a limited number of internal fixations performed for fracture patterns associated with significant risk of failure if definitive treatment is delayed for the 4–5 days that it takes injured personnel to arrive in CONUS (i.e., displaced femoral neck, peritrochanteric fractures, and displaced talar neck fractures) [28, 41, 43]. In a study of 47 patients with 50 fractures that underwent internal fixation in theater (16 of which were open fractures), 39 (78%) healed without apparent complications. Only one infection (with *Staphylococcus aureus*) was diagnosed in a patient with a closed medial malleolus fracture that was internally fixed with two cancellous screws. Notably, this was a highly select group, with the majority having had blunt trauma (68%), median ISS of 11, and mostly closed fractures [43]. Another small study evaluating 713 surgical cases during two OEF deployments at a hospital in Afghanistan between 2007 and 2010 evaluated both short- and intermediate-term outcomes of patients managed with internal fixation devices under the damage control protocol and found that with cautious selection, complication rates were acceptable [44]. Taken together, these studies concluded that internal fixation can be performed safely in the combat environment under highly select circumstances, though further study is needed to define the population in which this would be most appropriate [43, 44].

Traumatic wounds should not be definitively closed until serial debridements reveal stability. Within the first 72 h of injury, operative wound inspections should be performed every 24 h, with subsequent surgical timing based on wound appearance and persistent contamination [45]. Need for repeat debridement in theater

depends on a multitude of extrinsic (capability of evacuation, time to next surgical or definitive care facility, and patient load) and intrinsic patient factors (contamination of wound, location, risk of complications, presence of sepsis, perfusion of wound, and overall patient nutrition). During evacuation (Critical Care Air Transport Team [CCATT] evacuations for critically injured patients in the US system), repeat surgical evaluation and procedures are not possible. Patients may have prolonged recumbence, relative immobilization, and ongoing fluid resuscitation with peak tissue edema expected within 1–2 days post-injury. Therefore, a low threshold for release of compartments prior to patient transport is recommended when there is suspicion for potential compartment syndrome [28, 46–48].

For retained extremity metal fragments, conservative management with preemptive therapy of a single dose of a first-generation cephalosporin is recommended if the following wound characteristics are present: entrance/exit wound sites <2 cm, no high risk cause (i.e., mines), no bone or joint involvement, no breach of pleura or peritoneum, no major vascular injury, and not frankly infected [17, 41].

11.2.3 Unique Injury Patterns to OIF/OEF: The Dismounted Complex Blast Injury Pattern

A newly described injury pattern has become central to the casualty care of those critically wounded during OIF and particular OEF. Initially noted through the summer and fall of 2010, peaking in October, the Joint Theater Trauma System (JTTS) identified a new trend of devastating injuries characterized by proximal lower-extremity amputations associated with pelvic, genital, and spine injuries [8]. This DCBI pattern consists of (generally proximal) bilateral lower-extremity amputations with associated pelvic/perineal injuries. It is frequently accompanied by upper-extremity injuries (most commonly left sided due to weapon carrying stance during injury, but may be bilateral) as well as thoracoabdominal or neuraxial injuries. These patients often have additional complicating injuries including vascular, associated genitourinary, and (possibly occult) rectal injuries. These devastating injuries are associated with high morbidity and mortality. They are among the most challenging cohorts of surgical patients—from initial management to definitive reconstruction. Clinical Practice Guidelines (CPGs) have been established for the management of these patients, are available and updated online (https://jts.amedd.army.mil/index.cfm/PI_CPGs/cpgs), and should be referenced [45].

Because of the severity of injuries in patients with DCBI, they typically arrive critically injured shortly following injury. CPGs for damage control resuscitation and whole blood transfusion guide initial management and are outside the scope of this review [45, 49, 50]. Unfortunately, no longer unique to the war-wounded is the importance of triage in the setting of multiple or mass casualty scenarios. Initial operative goals are control of hemorrhage and contamination, which is best achieved by a team of general and orthopedic surgeons working simultaneously if possible.

Ideally, one team of surgeons works on proximal hemorrhage control and intra-abdominal injury management, a second team focuses on amputations, and a third team (if needed and available) addresses upper-extremity injuries. Initial orthopedic involvement is to ensure that extremity hemorrhage is controlled (with tourniquets or if needed by proximal vascular control in the abdomen or extraperitoneal space with pelvic packing). Reevaluation of field-placed tourniquets is imperative, as after initial volume resuscitation patients can bleed through in-place field tourniquets [42, 45]. Ten percent of single amputees and 39% of traumatic above-knee double amputations had associated pelvic fractures, so these casualties should be assumed to have unstable pelvic fractures until proven otherwise. Pelvic binders are routinely applied before evacuation. In these patients, speed is critical, and it is vital to assure adequate hemorrhagic control and timely wound debridement. Once bleeding is controlled, the binder should be replaced by an external fixator [42]. If possible, external fixation of long bone fractures can be accomplished during the index procedure. Small bone and joint fractures can also be addressed if the patient remains stable. However, these can also be performed afterward with splinting in the intensive care unit or during subsequent surgeries [45].

The extensive soft tissue damage and contamination associated with DCBI wounds is another challenge and requires aggressive surgical source control [8, 35, 45]. IED blast injuries may be associated with propulsion of contaminants along tissue planes to areas remote from skin disruption, making initial determinations of the zone of injury and need for tissue removal challenging [28]. Irrigation and debridement to remove gross contamination and devitalized tissues is the first step and should be done as soon as possible. Normal saline or sterile water is preferred (though potable water can be used if needed). CPGs call for 3, 6, and 9 L of irrigation fluid for type I, II, and III fractures, respectively. Low pressure (less than 14 PSI) is recommended due to evidence that pulse lavage is associated with higher rebound bacterial counts [17, 27, 32, 41]. Because of later coverage challenges, salvaging healthy tissue for flaps is paramount. However, it is imperative to avoid leaving marginally viable tissue behind which can serve as a nidus for infection and invasive fungal wound infection (IFI [which will be covered later in this chapter]) [45].

11.2.4 Perioperative Antimicrobials

Antimicrobial prophylaxis following combat trauma falls into two main categories: point-of-injury antimicrobials (as discussed above) and perioperative antimicrobials. Similar to civilian settings, guidelines recommend tetanus vaccine (and immune globulin if prior tetanus vaccination status is not adequate) and antimicrobial prophylaxis as soon as possible and ideally within 3 h of injury [51–53]. There is currently general consensus on the utility of short-course antistaphylococcal coverage for antimicrobial prophylaxis (i.e., cefazolin in the US and amoxicillin/clavulanate in the UK militaries, respectively). Unfortunately, current civilian guidelines differ in interpretations of the same literature, with the EAST guidelines recommending

addition of aminoglycosides for type III open fractures [53]. The Surgical Infection Society and Combat-related Extremity Injury guidelines recommend only high-dose cefazolin for prophylaxis, largely citing insufficient evidence for enhanced gram-negative coverage and concerns about selection of more resistant pathogens and unpredictable susceptibility of those organisms isolated from infections [32, 51, 52].

Unsurprisingly, with a history of conflicting recommendations, prior evaluations of practice patterns often showed broader and longer perioperative antimicrobial use. While the British Association of Plastic, Reconstructive and Aesthetic Surgeons/British Orthopaedic Association recommended antibiotic prophylaxis be limited to 3 days, in practice antibiotics were often continued until the treating surgeon feels the wound is free from signs of infection and "regarded as healthy" in one UK study [54]. In the US system, perioperative antibiotic prophylaxis with cefazolin and gentamicin was started in the OR and often continued until wound coverage in a cohort of open femur fractures [55]. The Joint Trauma Service CPG for Infection Prevention in Combat-Related Injuries has published injury-based recommendations on both perioperative and point-of-injury antimicrobial prophylaxis (Table 11.1) [56]. These recommendations emphasize the goal of prevention of early post-traumatic infections including sepsis and the use of the narrowest spectrum and duration required to attempt to minimize risk of multidrug-resistant bacteria [17, 45, 56, 57].

11.2.5   Local Wound Care and Antibiotic Delivery

Wounds without evidence of infection are recommended to be closed at approximately 5 days if technically possible [41]. Damage control orthopedics in combat-injured personnel commonly entails evacuation of injured patients across thousands of miles with open wounds. Initial recommended wound dressings include moist-to-dry, Dakin's soaked gauze, antibiotic bead pouches, or negative pressure wound therapy (NPWT) with reticulated open cell foam or moist gauze [45].

NPWT has been widely adopted in civilian trauma and has been shown to decrease time to wound coverage, with indirect effects on decreased wound infections. There is data from contaminated open fractures in animal models which show reduced bacterial counts and wound edema. Notably, this was primarily seen in *Pseudomonas* wound models and not seen in subsequent work with *S. aureus* [19, 58]. NPWT has also become standard of care for management of combat-injured patients at military treatment facilities. While it has been shown to be feasible during aeromedical evacuation, it is yet to have prospective trials showing decreased infection rates [32]. In one report, authors describe nearly universal adoption of NPWT during OIF (from 46% in March of 2003 to 90% of admitted wounds in 2005). They report the outcomes of 68 patients with large, complex wounds with extensive soft tissue and bony defects (55% having suffered blast injury). Through a combination of aggressive surgery and antimicrobials, antibiotic-impregnated beads, and NPWT, the authors reported limb salvage rates as high as 94%. However, they also noted acute and chronic osteomyelitis rates of 24% and 2%, respectively

(with a predominance of *Acinetobacter baumannii*) [59]. The biggest concerns with the use of NPWT in the combat-injured remain the potential for technical failure in the setting of power outages and subsequent anaerobic environmental conditions as well as potential lack of efficacy against *S. aureus* [19, 27, 60–62].

Current guidelines do not offer recommendations on the use of antibiotic-impregnated beads in the combat zone because of inadequate data in that challenging environment, but do recommend consideration of their use in the event of delayed evacuation [41]. One study retrospectively compared the performance of NPWT to antibiotic bead pouches for blast injuries in 12 matched patients. This study showed better outcomes with the use of the bead pouches with delayed primary closure at an average of 8 days compared to 12 days in those with NPWT. Additionally, those with NPWT also required four returns to the OR for infectious complications, all of which revealed growth of methicillin-resistant *S. aureus* (MRSA) which is particularly concerning with the prior animal data showing potential decreased efficacy of NPWT with *S. aureus*. Estimated costs were also $1000 more per treatment in the NPWT group compared to the antibiotic bead group [60]. Despite the results in this small study, the practicality of antibiotic bead pouches during aeromedical transport and serial debridements frequently required for combat-injured patients remains a technical challenge [41].

11.2.6 Infection Control and Prevention

In 2004, the first publications documenting rising issues with multidrug-resistant (MDR) bacteria, especially MDR *A. baumannii* complex infections in combat-injured patients led to investigations of the source of this outbreak [3, 63–68]. Ultimately, these investigations revealed nosocomial transmission related to a reservoir of host nation patients with prolonged hospitalizations, higher rates of pre-injury MDRO colonization, and environmental contamination [3, 32, 69–71]. *A. baumannii* complex has proven in the past to be especially problematic in the war-wounded, primarily related to its ability to survive for prolonged periods in hospital environments, nosocomial transmission, and ability to acquire antimicrobial resistance [19].

With operational theater shifts from Iraq to Afghanistan, MDR gram-negative infections transitioned from predominantly *A. baumannii* to extended-spectrum beta-lactamase-producing Enterobacteriaceae. This was likely related to a combination of increased pre-injury colonization, geographical differences in local national colonization, and accumulation of antimicrobial selection pressures along the chain of medical evacuation [3]. Thus, the importance of strong infection prevention and antimicrobial stewardship programs in the deployed environment cannot be overemphasized. Deployment of infection control teams over the last 10 years has identified similar themes including need for pre-deployment training in those assigned to infection control roles, microbiology support, environmental disinfection support, use of standardized procedures and guidelines, and antimicrobial stewardship [32].

With the lessons learned from the prior conflicts, the Infection Prevention in Combat-Related Injuries CPG recommends cohorting of "long term" (host nation patients, stays longer than 72 h) and "short term" (US personnel, less than 72 h stays) in deployed facilities to reduce the risk of cross-contamination with MDROs. Additionally, enhanced precautions should be used as clinically indicated (either contact for suspected community-acquired MRSA in presence of US personnel with SSTIs associated with abscesses or furuncles or enhanced contact precautions for those suspected of having *Clostridioides difficile*-associated diarrhea). ICU patients should undergo daily chlorhexidine gluconate bathing which has been associated with reductions in vancomycin-resistant enterococci, MRSA, as well as gram-negative bacteria including MDROs [56]. Currently, all injured soldiers admitted from deployed operations to CONUS facilities are placed in contact isolation, and screening swabs are collected to evaluate for MDR gram-negative and MRSA colonization. If all admission screening cultures are negative for MDROs, the soldier is then removed from contact isolation [63].

11.3 War Wound Infections

11.3.1 Introduction

Severe war wounds are associated with multiple risk factors for subsequent infection including devitalized tissue, gross contamination, foreign bodies, and fluid collections [25]. The fundamental principles of management of these wounds (washouts, topical therapy, bandaging, and stabilization) were described as early as 4000 years ago in Sumerian carvings and remain central [25, 72]. Colonization is defined as the presence of non-replicating bacteria on the wound surface that don't initiate a host response. Wound infection is generally defined as invasion and multiplication of microorganisms in a wound resulting in tissue injury and host immune reaction [19].

11.3.2 Epidemiology

Comparisons of infectious complications of war wounds between current and prior conflicts are challenging because of the relative paucity of prior long-term follow-up [3]. Additionally, because of the differences between mechanisms of injury and medical capabilities available at initial triage, comparison with civilian literature can be challenging as well. A study which sought to compare civilian and deployed military medical facilities described the patient populations and outcomes of the 228th Combat Support Hospital (CSH) in Tikrit, Iraq, to that of the trauma registry at Oregon Health & Science University between December 2004 and November 2005.

While there were some methodologic limitations, including the fact that the Combat Support Hospital specialized in the care of nonbattle injury, lacked a computed tomography (CT) scanner, had only a single general surgeon (as compared to the three surgeons typically available at US Army Forward Surgical Team), and few surgical subspecialists, this study represented the first attempt to compare the care delivered at a Combat Support Hospital to that at a civilian level I trauma center. Major points included the fact that those admitted to the CSH were primarily injured by high powered penetrating mechanisms as opposed to blunt mechanisms in the civilian setting. Trauma patients in the civilian setting were noted to be older and have a higher ISS. While the percentage of abdominal, thoracic, and vascular procedures were similar between the two settings, the war-wounded had more soft tissue procedures. Reassuringly, there were no differences in mortality between the civilian and military setting [73]. With these concerns for increased infections in combat-wounded patients and difficulty comparing populations with disparate mechanisms of injury and severity, a study at the only level I trauma center in the DoD revealed similar rates of infectious complications in noncombat-trauma patients cared for at that institution compared to those at other trauma centers in the USA [74].

Looking at early infectious outcomes during OIF/OEF within the TIDOS study, 45% of infections diagnosed prior to initial hospital discharge were SSTIs. At a patient level, 20% of patients had an SSTI during their initial hospitalization. There was a median of 9 days (IQR 5–17) between initial injury and diagnosis of first SSTI [15]. Studies have identified various measures of injury severity, including injury severity score, more than four injuries, blast as the mechanism of injury, volume of blood transfusions, sustaining an open or soft tissue injury, use of prophylactic antibiotics within 48 hours of injury, having an external fixator, use of tourniquets in the field, bone loss, sustaining an amputation, first documented shock index ≥ 0.80, and admission to the ICU as risk factors for infectious complications following combat-related injuries [71, 75–78]. As the theater of operations transitioned from Iraq to Afghanistan, increased infectious complications were noted. However, analysis revealed that this was related to increased injury severity and more blast-related trauma in Afghanistan [23, 76].

Extending this follow-up past discharge from initial hospitalization (which has little data from prior conflicts with which to compare) revealed continued infectious complications. Of 1006 patients enrolled in the TIDOS cohort between 2009 and 2012, there were some notable differences between those who did and did not enroll. However, overall the cohort enrollment was almost 50% and representative of those with severe injuries (ISS ≥ 16 and mechanical ventilation required in more than a third). Of enrolled patients, 318 (32%) had infections diagnosed following initial hospital discharge, of which SSTIs accounted for 66%. Of these patients, 183 (58%) had only one infection, 76 (24%) had two infections, 32 (10%) had three infections, and 27 (8%) had at least four infections. SSTIs occurred a median of 126 days following discharge. Of the 357 patients in the cohort who had infections diagnosed during the inpatient hospitalization, 160 (45%) had infections diagnosed during the follow-up period as well. Sustaining an amputation or open fracture, having an infection during initial inpatient hospitalization, and use of an

anti-pseudomonal penicillin for at least a week were independently associated with an increased risk of development of an extremity wound infection during follow-up, while shorter hospitalizations (15–30 days) were associated with reduced risk [16].

A recent study evaluating even more long-term infectious outcomes following wounded service members through transition to Veterans Affairs (VA) care revealed that SSTIs and osteomyelitis remain the predominant infectious complications following combat injury. Overall, of the first 337 TIDOS enrollees who entered VA healthcare, 38% had a new trauma-related infection after initial hospital discharge, and 29% occurred after the patient left military service. The most common infections after initial hospital discharge were SSTIs (68%) and osteomyelitis (13%) at a median of 829 and 81 days following hospital discharge, respectively. This study emphasizes not only that the burden of infectious complications following war injuries lasts long past initial hospital discharge but also the importance of interagency research collaboration [14].

11.3.3 Microbiology

Initial microbiology at time of injury of recent combat wounds reveals predominantly usual skin flora, including coagulase-negative staphylococci and *S. aureus* [32, 78, 79]. Without clinical evidence of infection, wound cultures are not recommended at the time of debridement as these cultures don't predict future infecting pathogens [19, 80].

The microbiology of early infections reflected the theater of operations in recent conflicts, with the majority of gram-negative infections during OIF being MDR *A. baumannii* (70% of the cases), *K. pneumoniae*, and *E. coli*. With transition to OEF, *A. baumannii* was replaced by extended-spectrum beta-lactamase-producing *Enterobacteriaceae*, most commonly *E. coli* as the predominant gram-negative pathogen without evidence of clonality [32, 81–85]. Microbiology of infections in complex extremity wound infections (CEWIs) remains similar to prior analyses with initial hospitalization CEWIs mainly secondary to gram-negative pathogens [16].

SSTI diagnoses during initial hospitalization were mostly polymicrobial with gram-negative bacteria isolated from CEWIs from 50% and 90% of monomicrobial and polymicrobial infections, respectively. The most frequently isolated gram-negative bacteria were *E. coli, Enterobacter* spp., *Pseudomonas* spp., and *Acinetobacter* spp. Gram-positive bacteria (most frequently *Enterococcus* spp.) were also isolated in 74% of polymicrobial wound infections. Reassuringly, only 14% of gram-positive SSTI organisms were MDR. However, almost half of gram-negative organisms associated with SSTI diagnoses were MDR (primarily driven by *E. coli* and *A. baumannii* which were up to 75% and 95% MDR, respectively, depending on the study). Molds/yeasts and anaerobes were isolated from 40% and 17% of polymicrobial infections. Thirty-eight percent of infected wounds had growth of a combination of bacteria with mold and/or yeast. Patients with polymicrobial CEWIs had higher ISS, more traumatic amputations, and more frequently

required ICU admission. In comparison to those with confirmed and suspected CEWIs, those with colonized wounds were largely monomicrobial (58%) and primarily identified molds [15, 86]. Following initial hospitalization, long-term follow-up reveals predominantly *S. aureus* (26% of SSTIs), of which 31% were MRSA [16].

The challenges of MDR infections in war-wounded are not unique to military personnel. Recent publications from Doctors Without Borders describe complications of civilians with acute war injuries obtained during the Syrian armed conflict cared for within the Ministry of Health Hospital in Ar Ramtha, Jordan. They were managed according to the International Committee of the Red Cross (ICRC) war surgery protocol with surgical wound debridement, prophylactic narrow-spectrum antibiotics for 48–72 h, and delayed primary closure of wounds at 3–5 days if possible. Of 457 civilian men admitted following blast and gunshot injuries from the Syrian armed conflict, clinical signs of infection were noted in 18%, and 11% were confirmed with culture. The most common bacteria were *S. aureus* (73% MRSA), *Pseudomonas* spp. (17% MDR), *K. pneumoniae* (82% MDR), *Enterobacter* spp. (78% MDR), *E. coli* (100% MDR), *Proteus* (63% MDR), and *Acinetobacter* spp. (100% MDR). While most of the MDR *Enterobacteriaceae* remained susceptible to carbapenems, most of the *Acinetobacter* were also carbapenem-resistant [87].

11.3.4 Diagnosis

Diagnosis and treatment of orthopedic injuries following combat injury are similar to those in any other traumatic situation. SSTIs are largely diagnosed via clinical appearance of the wound [32]. Features suggestive of infection are increasing pain, erythema, and heavy discharge from the wound, which may or may not be associated with systemic features like fever and elevated inflammatory markers [19]. Wound infections are classified as deep or superficial with depth and extension defined during surgical debridement [32].

11.3.5 Complications

Besides the obvious complication of additional procedures and antimicrobial administration needed for treatment of deep wound infections, 43% of those in the TIDOS cohort who had extremity wound infections during initial hospitalizations had SSTIs or osteomyelitis diagnosed in follow-up (of which 19% were recurrent infections at the same site and 34% were incident infections). CEWI as an inpatient had a relative risk of 2.25 for developing a delayed CEWI during follow-up [16]. In civilians hospitalized for combat injuries associated with the armed conflict in Syria, those with infection had a higher amputation rate (22% vs 9%), more procedures (12 surgeries compared to five), and longer duration of hospitalization

(77 days compared to 35 days) than those without infections [87]. In the TIDOS study, of the 318 patients with infections diagnosed following discharge from the hospital (76% SSTIs, 23% osteomyelitis, 11% UTIs), 23% were hospitalized and 40% required surgery as a result of the infection [16].

With studies showing prior wound infections as a risk factor for future wound infections, a recent, small study sought to evaluate the role of biofilms in persistent wound infections from combat-trauma patients. Biofilm is produced when bacteria are able to grow on a solid surface and avoid host immune responses by two main mechanisms: prevention of penetration of antimicrobials to the site of infection and cessation of replication (rendering those bacteria resistant to currently available antimicrobials). This study evaluated the most common etiologies in CEWI from combat-injured patients enrolled in TIDOS (*S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. coli*) collected from cases (SSTI wounds with the same organism persistently isolated for 14 days) and controls (SSTI wounds with non-recurrent growth of bacteria). They found that persistent isolation of the same organism was associated with biofilm formation (odds ratio [OR] of 29.5), MDROs (OR 5.6), transfusion requirements (OR 1.02), number of OR visits (OR 2.1), sites of infection other than the lower extremity (primarily groin and pelvis; OR 5.5), and polymicrobial infection (OR 69.7). Interestingly, presence of hardware was not associated with persistent isolation of the same organism, but the study was small and likely underpowered to detect this [88].

11.3.6 Therapy

Due to the frequency of MDR isolation and polymicrobial nature of wound infections following combat-related injuries, once infection is suspected or diagnosed, empiric therapeutic antimicrobials should be broad spectrum. Antibiotics can then be narrowed once culture results are available. Similar to civilian literature, duration of antibiotics is determined by the depth and extent of infection, with superficial infections requiring little more than debridement. However, deep wound infections frequently require 1–2 weeks of antibiotics in combination with serial debridements [32].

11.4 Combat-Related Osteomyelitis and Orthopedic Device-Related Infections

11.4.1 Introduction

In contrast to civilian open tibial fractures, in which the most common mechanism of injury is blunt trauma (motor vehicle accidents, falls from height, and pedestrians struck by motor vehicles), combat-associated open fractures are most often due to

penetrating trauma from blasts or gunshot wounds, have an "outside-in" mechanism, are frequently grossly contaminated, and are associated with other wounds [45, 89]. A study of 850 civilian vs 115 combat-associated open tibial fractures revealed that those associated with combat trauma were severely injured, more likely to be hypotensive, secondary to penetrating trauma, and have higher rates of amputation for Gustilo-Anderson IIIB and IIIC fractures. These worse outcomes were thought to be primarily related to blast (and particularly IED) mechanism of injury which was seen in the majority of military patients and was responsible for most of the Gustilo-Anderson grade III injuries, Mangled Extremity Severity Score (MESS) ≥7, and amputations. While the MESS did not predict ultimate need for amputation in either group, a MESS of ≤7 was associated with potential for limb salvage. In the military group, the elevated MESS were driven primarily by presence of shock and ischemia, and the only single MESS factor predictive of amputation on univariate and multivariate analysis was limb ischemia. However, when limb salvage was attempted in the presence of limb ischemia, it was successful in a majority of cases, despite a MESS ≥ 7. Overall, the presence of ischemia from vascular injury was the most sensitive predictor for future amputation need, but when limb salvage was attempted, it was largely successful. As such, if hemostasis and contamination can be controlled and rapid vascular repair or shunting can be performed, limb salvage may be possible [90].

11.4.2   Epidemiology

Rates of infection following open fractures in US and UK military cohorts have revealed rates of osteomyelitis of 8–25% and deep infections of 25%, respectively [16, 77, 89, 91–93]. Osteomyelitis recurrence rates as high as 18% have been described in patients with a diagnosis of osteomyelitis at the same site during initial hospitalization [16, 92]. Because of differing methodologies, median time to diagnosis of osteomyelitis ranged from 15 days to 10 months [16, 93]. In general, UK military management of open fractures mirrors that in the civilian world, with the exception that initial management often includes traction and casting (rather than external fixation as is seen in the US military). Additionally, with blast trauma and severe tissue lost related to mechanisms of injury associated with trauma, extensive wounds also allow access to the fracture, so higher rates of plate fixation were used in the UK series compared to IMN (typically unreamed solid IM nails are used to reduce surgical insult and OR time) [54]. In comparison the US military, as detailed above in the DCBI descriptions, primarily uses external fixation for initial management until evacuation to CONUS hospitals allows definitive internal fixation [17, 28, 32, 55]. It can be challenging to compare the rates of infections between the US and UK experiences because the studies had different definitions of osteomyelitis, timing of osteomyelitis diagnosis, and primary mechanisms of injury (US 71% blast compared to 46% in UK) [16].

Overall, risk factors for osteomyelitis identified in various studies include Gustilo-Anderson classification and Orthopaedic Trauma Association Open Fracture Classification for muscle loss and dead muscle, earlier time of injury (presumably related to higher use of high-pressure irrigation and prior to transition to NPWT and use of damage control resuscitation and transfusion CPGs), evidence of gross contamination of open fractures (via antibiotic bead use or positive initial screening cultures on CONUS admission), IED blast injury, and foreign body contamination with implant at the fracture site [55, 91, 93, 94].

11.4.3   Microbiology

Similar themes to those noted during evaluation of CEWI are noted in evaluation of microbiology of osteomyelitis in combat-trauma patients, with a predominance of gram-negatives in early diagnoses transitioning to gram-positives (and primarily *S. aureus*) in late and recurrent infections [19, 78, 95]. Early evaluation of the JTTR, during two phases of OIF/OEF (2003 and 2006), of which 96% of patients were evacuated from Iraq, again showed the majority of infections associated with gram-negative organisms. Unfortunately, as this study used primarily ICD-9 coding for microbiology, it lacked the granularity to evaluate for specific microorganisms of interest, especially *A. baumannii*, and long-term follow-up [23]. In one retrospective study evaluating outcomes at a single institution from combat-trauma patients from OIF, osteomyelitis was diagnosed in 15% of casualties admitted to the orthopedic service. Gram-negatives (primarily *Acinetobacter* spp., *K. pneumoniae,* and *P. aeruginosa)* were more likely to be isolated during initial osteomyelitis diagnoses, compared to *S. aureus* (both methicillin-susceptible *S. aureus* [MSSA] and MRSA) which were more likely to be isolated from recurrences [55, 92]. In an evaluation of the microbiology of severe open tibial fractures from combat, early surveillance swabs yielded primarily gram-negative species (in 91% of cases, with 26% gram-positives and 34% polymicrobial). Notably, surveillance cultures did not predict later microbiology of infection, of which only 7% was the culture the same as the initial surveillance culture. Later deep infections were more likely to be gram-positive (68%; 52% gram-negative and 24% polymicrobial) [89]. In comparison, a TIDOS study evaluating longer-term follow-up revealed that 24% osteomyelitis cases diagnosed after initial hospitalization were methicillin-resistant *S. aureus*. Coagulase-negative staphylococci were recovered from an additional 20% of osteomyelitis cases, with *E. coli* linked to only 7% of osteomyelitis (of which 17% were MDR), *P. aeruginosa* from only 8% (8% of which were MDR). Notably, *Acinetobacter* was not isolated from any osteomyelitis cases [16].

11.4.4 Diagnosis

Osteomyelitis in the combat-injured is diagnosed similarly to other contexts, at times obvious due to necrotic bone, abscess, and/or sequestrum formation, or inferred based on deep wound infections contiguous with the bone or hardware [32]. Multiple cultures should be obtained to maximize yield and interpretation. While there are no guidelines for orthopedic device-related infections, many recommendations for diagnosis and management are extrapolated from the prosthetic joint infection literature and guidelines. Yield of cultures is highest if obtained prior to antibiotics, but perioperative prophylactic antibiotics should not be withheld for this purpose [32, 96, 97].

11.4.5 Complications

Deep wound infections and osteomyelitis have been associated with decreased return-to-duty rates, hospital readmissions, and failure of limb salvage [32]. In a US cohort of open femur fractures, those with infectious complications had a mean time to union of 6.5 months compared to 4.6 months in those without infection. Of eight diagnosed with deep infections requiring serial I&Ds and parenteral antibiotics for 4–6 weeks, five required intramedullary nail removal to clear the infection. All patients with infectious complications were ultimately successfully treated without recurrence [55]. In a similar cohort from the UK military, of those with open femur fracture infections, none healed without further surgery [54]. In an evaluation of 115 wounded soldiers with type III open tibia fractures, diagnosis of any infection (and osteomyelitis) was associated with a lower rate of return to duty (24% and 10%, respectively) compared to those without infectious complications [98]. Those with severe open tibial fractures without infection had an amputation rate of 15.5% and time to union of 8.6 months compared to those with deep infection or osteomyelitis who had more than double the amputation rate (34.3%) and a time to union of 11 months [89]. In another evaluation of open tibial fractures, osteomyelitis cases had significantly longer time to radiographic union (median 210 vs. 165 days) compared to those without osteomyelitis [91].

11.4.6 Therapy

Unfortunately, because of the nature of fractures in the combat-injured, many are associated with orthopedic hardware. Ideally, due to the nature of biofilm and difficulty with eradication of bacteria within biofilm, the involved hardware would be removed or replaced [32]. However, this can be challenging with the nature of injuries, a series reporting on damage control orthopedics outcomes from war-wounded

reported that of infected nails, 70% ultimately had union with nail retention in 57% [93]. Overall management of osteomyelitis and ODRIs complicating war wounds are similar to those in the civilian setting and frequently require 4–6 weeks of antibiotic therapy and may be even more prolonged in the setting of orthopedic hardware [32]. Because of the cost and complications associated with prolonged IV antibiotic therapy, there is growing interest and literature to support the potential use of highly bioavailable oral regimens for treatment of bone infections, though the pivotal study describing this strategy had high rates of surgical source control, which can be exceedingly challenging in many complicated war wounds [99].

11.5 Combat-Related Invasive Fungal Wound Infections

11.5.1 Introduction

As initial recognition of the DCBI pattern was being formalized, the initial descriptions of the IFI outbreak were also being reported [3, 11, 32, 45, 100–104]. For those who survived initial DCBI, late complications included sepsis, hospital-associated infections, multisystem organ dysfunction, and a new phenomenon of recurrent wound necrosis despite multiple surgical debridements [45]. IFIs are devastating infections associated with increased mortality, morbidity (amputations, hemipelvectomies), and prolonged hospitalizations for survivors [105].

Combat-related IFI has been defined as the presence of a traumatic wound(s), recurrent necrosis following at least two consecutive surgical debridements, with laboratory evidence of fungal infection (culture with mold and/or histopathology with evidence of fungal tissue invasion) [11, 103–105]. Various further classifications have been included in studies, most commonly dividing cases up by certainty of diagnosis of IFI using combinations of culture and histopathology: proven IFI (mostly requiring angioinvasive fungal elements on histopathology), probable IFI (fungal elements identified on histopathology without angioinvasion), possible IFI (cases in which fungal culture revealed growth of mold, but histopathology was negative for fungal elements), and unclassifiable IFI (cases in which fungal culture isolated mold, but no histopathology was performed) [104].

11.5.2 Epidemiology

Following recognition of the IFI cases, the Joint Trauma System, in collaboration with TIDOS, launched an investigation which revealed the most common epidemiologic risk factors associated with IFI as dismounted blast injury, above-the-knee traumatic amputations, extensive pelvic/perineal injuries, and receipt of massive packed red blood cell (PRBC) transfusions (≥20 units in the first

24 h) [11, 105]. Following the outbreak identification, a CPG was implemented at LRMC in early 2011 to screen for IFI with culture and histopathology in high-risk patients. Over the next 6 months, 61 patients were screened and 30 IFI cases identified. Despite evidence to suggest higher severity of illness in those screened following CPG initiation, time to IFI diagnosis (3 days vs. 9 days) and initiation of antifungal therapy (7 vs. 14 days) were significantly decreased following the initiation of the CPG. Additionally, pre-IFI CPG cases were more likely to be associated with angioinvasion on histopathology than those identified during the CPG period (48% vs. 17%, respectively). There was also a nonsignificant decrease in crude mortality from 11.4% to 6.7% following CPG initiation. However, to complicate matters, it was also noted that mold was frequently isolated from these screening cultures and did not predict subsequent IFI [100]. Because of the frequency of blast injury, severe injuries, and isolation of molds from these contaminated wounds and the morbidity associated with management of these wounds (serial debridements and combination antifungal therapy), further evaluations were completed to attempt to better refine risk factors. Ongoing case finding confirmed prior JTS IFI CPG risk factors with multivariate analysis identifying blast injuries (odds ratio 5.7), dismounted blast injury (OR 8.5), above-the-knee amputations (OR 4.1), and large-volume packed red blood cell transfusions (PRBC>20 units; temporary related immunosuppression and iron overload) within the first 24 h (OR 7) as independent risk factors [102].

While the prior definitions were based on initial cases identified, a recent comprehensive review of 1932 patients evaluated at participating TIDOS hospitals, with 720 (37%) with penetrating wounds and operative cultures and/or histopathology sent, revealed that 246 (34%) met criteria for laboratory evidence of fungal infection. Retrospective analysis divided these cases into those with wounds meeting IFI criteria, wounds highly suspicious for IFI (wounds that did not meet criteria for IFI, but had signs and symptoms of deep SSTI and received at least 10 days of antifungal therapy or required a proximal amputation), and wounds with low suspicion for IFI (wounds that did not meet criteria for IFI, did not meet criteria for deep SSTI, or met criteria for a deep SSTI due to bacteria [could have antifungal therapy <10 days] but with laboratory evidence of fungus [positive fungal cultures, histopathology, or both]). Ultimately, demographic and injury characteristics were unable to stratify risk of IFI, high-suspicion, or low-suspicion wounds—all groups were primarily composed of men who were critically injured by blasts on foot patrol with massive blood transfusions which resulted in great practice variation in those meeting criteria. As such, these epidemiologic risk factors served as poor markers for those who needed more intensive surgical management and systemic antifungals. However, from a wound level, those wounds without ongoing necrosis, lacking persistent fungal isolation, and without evidence of deep SSTI were at low risk for IFI and recommendations made were to monitor these closely in this patient population [106].

11.5.3 Microbiology

Early evaluations of the microbiology of combat-associated IFI revealed the diversity of associated fungi isolated from cultures. These were challenging to interpret in the setting of high rates of polymicrobial infections including bacteria (not uncommonly MDR), *Candida* spp., and multiple orders of molds [3, 11, 85, 86, 107, 108]. Only 1% of bacterial cultures collected within the first 14 days of injury in those assessed for IFI were negative. Notably, *Acinetobacter baumannii* and MDROs were more frequently isolated from patients with IFI than from patients in high- or low-suspicion wounds [106].

As the polymicrobial nature of these wound infections was clarified, isolation of the order *Mucorales* was noted to be associated with worse outcomes, as has been previously reported in necrotizing fungal wound infections following natural disasters [107, 109, 110]. A recent study, which attempted to use a pragmatic approach based on wound appearance, microbiology, and epidemiologic risk factors to evaluate which wounds needed more aggressive surgical debridement and empiric systemic antifungals, revealed that while epidemiologic factors had relatively low specificity for definitive IFI, microbiology was more helpful. Among 413 wounds with documentation of fungal infection from combat-wounded service members, 97% had cultures submitted (of which 11% were negative). Fungi of the order *Mucorales* were more frequently isolated from IFI (39%) and high-suspicion wounds (22%) than low-suspicion (9%) wounds. *Fusarium* spp. were also more commonly isolated from IFI wounds than low-suspicion wounds, but at a lower rate (17% and 4%, respectively) [106].

11.5.4 Diagnosis

Overall, the diagnosis of IFI requires a recognition of epidemiologic risk factors (traumatic inoculation of environmental debris into high-risk wounds). In this setting, if wounds show recurrent necrosis, empiric systemic antifungals and aggressive debridements should be initiated while awaiting diagnostic studies. This requires a multidisciplinary approach with collaboration between surgery, infectious diseases, and laboratory support with both cultures and histopathology [11, 32, 100, 101, 103–106, 111]. A web-based clinical decision tool has been developed by the Surgical Critical Care Initiative (SC2i) and the TIDOS project to assist healthcare providers in determining the risk of IFI (available at http://www.sc2i.org/ificdss). For patients with at least three IFI risk factors (Table 11.2), tissue biopsy in the operating room should be obtained at the time of wound exploration (after initial surgical debridement) once the casualty has been evacuated from theater and repeated on subsequent evaluations if there are persistent fevers or wound necrosis concerning for IFI. Tissue samples should be obtained from each lower extremity in patients with bilateral lower-extremity amputations. Samples should be obtained from both compromised muscle and adipose tissue, as well as other sites at the

Table 11.2 IFI risk factors

1. Dismounted blast injury
2. Traumatic transfemoral amputation(s) or rapidly progressive transition from transtibial to through knee or transfemoral
3. Extensive perineal, genitourinary, and/or rectal injury
4. Massive transfusion: >20 units packed red blood cells within 24 h of injury

Adapted from Rodriguez CR, et al. Treatment of Suspected Invasive Fungal Infection in War Wounds, Mil Med, 183, 9/10:142, 2018

surgeon's discretion. At least one specimen should be obtained from the junction of viable and necrotic tissue. For each site sampled, it is critical to obtain both histopathology and culture (fungal and bacterial). This requires placement of each site's sample into two separate sterile specimen containers (to avoid loss of culture from placement in formalin for histopathology). More detailed descriptions of procedures within the DoD are available [105].

11.5.5   Complications

The morbidity associated with IFI in those who have already suffered devastating DCBI patterns cannot be overemphasized [11, 103, 106, 107]. IFI has been associated with high-level amputations (22% in proven and probable cases), including hip disarticulations and hemipelvectomies, and crude mortality rates of 9% [32]. Studies which have compared IFI wounds to non-IFI wounds (both with concomitant SSTIs and without) have revealed that IFI wounds were associated with longer time to wound closure than non-IFI controls with and without SSTIs [107, 112]. IFI wounds resulted in significantly more amputation revisions and more frequently required proximal revision of a functional amputation level (34% vs 13%, respectively). Particularly notable, transfemoral amputations with IFI were more frequently revised to hemipelvectomies or hip disarticulations. Complications requiring repeat surgery for either drainage or infection after wound closure were also more frequent in IFI wounds (50% compared to 20% control wounds) [112]. Even among those with IFI wounds, time to closure was significantly longer in those wounds with *Mucorales* growth compared to those with non-*Mucorales* growth (median 17 vs. 13 days) and required more OR visits (median of 10.5 and 9 OR visits respectfully) [107].

11.5.6   Therapy

Treatment of IFI is based on three key principles: early recognition of at-risk wounds with repeated debridements of infected and necrotic tissue, minimization of immunosuppression (i.e., avoiding malnutrition or excessive blood transfusions in the

combat-trauma population), and utilization of empiric broad-spectrum antifungals when there is a high suspicion of IFI (typically dual therapy of liposomal amphotericin B and a broad-spectrum triazole) [105]. Because of the time required for definitive fungal identification, MDR nature of some of the fungal pathogens associated with this diagnosis, and unclear penetration of both topical and systemic therapies into these high-risk wounds, the critical role of surgical source control of IFI in this population cannot be overemphasized. The patient should undergo surgical evaluation, wound washout, and debridement (if required) within 12–18 h of arrival to a deployed hospital with surgical capabilities. The role of topical antifungal therapy (Dakin's solution) remains unproven, but has not been shown to have adverse local or systemic effects, so is currently recommended as an adjunct. Options include Dakin's wound irrigations or Dakin's-soaked Kerlix dressing. Topical antifungal therapy with 0.025% Dakin's solution via installation vacuum dressing should be continued through the evacuation phase if possible. Because of the nature of medical evacuation and multiple handoffs throughout echelons of care, a standardized operative note for wound description using the Bastion Classification of Lower Limb Injury (Table 11.3) should be used [105].

On arrival to each hospital along the chain of evacuation and on arrival to a CONUS hospital if the concern for IFI remains, the patient should undergo operative exploration, washout, and debridement as needed within 12–18 h. Histopathology and microbiology specimens should be obtained as described above. If a significant amount of necrotic tissue is debrided, repeat debridement should be performed within the next 24 h and continue at least every 24 h until cessation of necrosis occurs. Topical antifungal therapy should be continued until the surgeon observes healthy granulation or histopathology and cultures are negative for fungal infection or colonization. Topical antibacterial and antifungal beads (composed of liposomal amphotericin B 500 mg, voriconazole 200 mg, tobramycin 1.2 g, and vancomycin 1 g) may be used in conjunction with vacuum instillation or dressings [105].

For patients with recurrent tissue necrosis following two consecutive debridements (not including the first two debridements in theater), broad-spectrum antifungal and antibacterial therapy should be started immediately and infectious disease consultation obtained. Because many IFI wounds grow more than one mold (and these molds can have intrinsic resistance to various agents), dual antifungal therapy

Table 11.3 Bastion classification of lower limb injury caused by improvised explosive device

Class of limb injury	Description
1	Injury confined to foot
2	Injury involving lower leg permitting effective below-knee tourniquet application
3	Involving thigh injury, preventing effective tourniquet application
4	Proximal thigh injury, preventing effective tourniquet
5	Any injury with buttock involvement

Adapted from Rodriguez CR, et al. Treatment of Suspected Invasive Fungal Infection in War Wounds, Mil Med, 183, 9/10:142, 2018

is recommended with liposomal amphotericin B and a broad-spectrum triazole. Most clinical experience has been with voriconazole in these infections based on the timing of the outbreak, but posaconazole and isavuconazole are also potential options. These wounds are frequently polymicrobial with bacterial coinfection and not infrequently MDR (as detailed above), so in addition to dual antifungal therapy, broad-spectrum antibacterials are recommended (e.g., vancomycin and merope- nem). Current recommendations are to stop systemic antifungal medications if the patient remains clinically stable and wound remains viable/clean for 2 weeks with- out evidence of other metastatic foci of infection. Wound closure should not occur until the wound is clean, contracting, and granulating [105].

11.5.7 Prevention

Although preventive strategies have not been clearly identified, early and aggressive debridement of devitalized tissue and removal of debris are thought to be critical [105]. Additionally, ability to predict the environmental factors associated with these infections in the event of a new theater of conflict could help identify future high-risk wounds. A study evaluated the environmental conditions in Southern Afghanistan (which was the center of the combat-related IFI outbreak) to that in Eastern Afghanistan (not associated with the IFI outbreak) to attempt to identify environmental characteristics to model risk for IFIs after traumatic injury in other areas. Multivariate analysis revealed that lower elevation, warmer temperatures, and greater isothermality were independently associated with mold contamination of traumatic wounds [113].

11.6 Research and Ways Forward

MDROs remain a major risk for our combat-injured personnel and victims of war- time violence, but that epidemiology is not fixed, as we have seen from the transi- tion from outbreaks of MDR *Acinetobacter baumannii* in OIF to the predominance of ESBL-producing Enterobacteriaceae during OEF. This emphasizes the impor- tance of continued work to improve diagnostics, surveillance, and treatment in the- ater [3, 32, 114–116]. Ongoing surveillance, antimicrobial stewardship, local antibiograms, and continuous process improvement are critical for continued improvements in care provided in the austere environment and CONUS facilities for these wounded personnel. Research is ongoing to attempt to validate closed systems which can analyze both speciation and susceptibility testing directly from clinical samples with minimal laboratory technical expertise required. Additionally, efforts for global resistance surveillance can contribute to increased understanding of the epidemiology of resistance in associated populations [114, 117].

Advancing diagnostics (both in theater and at home) continues to remain a central effort for the care of infections complicating war wounds. With knowledge that hospitals in the combat zone often lack sophisticated culture and susceptibility testing, but MDROs play a dominant role in combat-related infections, the use of molecular rapid diagnostic test (RDT) systems has been of particular interest. These are a potential future option if locally prevalent molecular resistance mechanisms are known. Unfortunately, currently available RDT bacterial resistance testing requires incubation and is not widely available on primary clinical sample materials [117].

With increased use and study of molecular methods for diagnosis of difficult to culture pathogens, we are beginning to understand the complexity of the acute and chronic wound microbiome. However, the significance of these pathogens and their role in wound healing, host immune and inflammatory responses, and subsequent infections is yet to be fully defined. For instance, a study of 124 wound samples from extremity injuries from combat-injured US soldiers revealed microbial targets in 51% of all wound samples, with *A. baumannii* being the most common. Notably, there were large discrepancies between wound cultures and their molecular results—with 34% of culture-negative wounds identifying at least one organism via molecular methods and 18% of cultured organisms not identified via molecular testing. Interestingly, while no association between culture status and subsequent wound failure was noted, *Pseudomonas* was detected at the wound level in 3% of healed wounds compared to 23% of wounds with failure. Additionally, an inverse correlation was noted with detection of Enterobacteriaceae in 30% of samples from healed wounds compared to 4% in those which failed. While *Acinetobacter* detection was not associated with wound outcome, detection of *Acinetobacter* plasmid pRAY (a plasmid associated with multidrug resistance) was detected in 15% of healed wounds compared to 41% of wounds with failure. Overall, the authors concluded that current bacteriology methods underestimate the complexity of wound microbiomes and fail to predict wound outcomes, so further research should work to elucidate the use of molecular techniques in this setting [118].

MDR gram-negative organisms play a predominant role in wound infections complicating war trauma, so novel antimicrobials for treatment of these challenging infections are necessary. Additionally, decreased time for identification of bacteria and resistance would allow decreased time between infection concern and ability to narrow antimicrobials, decreasing selection pressure and antimicrobial resistance [41]. Speed of diagnosis in fungal infections associated with combat trauma is also a crucial effort—not only with epidemiologic risk factors, identification of new geographic areas of risk, and prognostic efforts, but also with diagnostics [106, 107, 113]. Because the time and technical skill required for initial fungal identification and speciation can have significant complications in combat-related IFI with ongoing wound necrosis, serial debridements (resulting in more proximal amputations), and systemic antifungal exposures, studies are ongoing to evaluate the use of PCR in these wounds which could speed the diagnostic process [119].

Overall, research on infections complicating war wounds attempts to improve care from point of injury to late complications. The importance of ongoing efforts

to ensure systematic collection of data stretching from point of injury through long-term outcomes will allow continued improvements in the care of the war-wounded [3]. The translation of key findings from civilian literature to the combat-wounded and vice versa remains critical to ongoing progress.

11.7   Conclusion

With modern warfare not sparing civilians, porous international borders, and increasing use of intentional violence and suicide bombings, nonmilitary physicians are increasingly seeing complications of penetrating and war-related injuries [87, 120–123]. IFI has recently been described following a range of natural disasters, with similar presentations of recurrent wound necrosis and invasive fungal infections following traumatic inoculation of fungi [109, 110, 120, 121]. Additionally, with increasing survivability following prior fatal injuries, we are now seeing more patients with long-term sequelae of severe battle injuries. Efforts to systematically evaluate the long-term outcomes following these injuries are just in their nascent stages, so much work remains to be done [14].

References

1. Defense USDo. OIF/OEF Casualty Status. Available at: https://www.defense.gov/Newsroom/Casualty-Status/.
2. Belmont PJ, Owens BD, Schoenfeld AJ. Musculoskeletal Injuries in Iraq and Afghanistan: Epidemiology and Outcomes Following a Decade of War. J Am Acad Orthop Surg 2016; 24(6): 341–8.
3. Blyth DM, Yun HC, Tribble DR, Murray CK. Lessons of war: Combat-related injury infections during the Vietnam War and Operation Iraqi and Enduring Freedom. J Trauma Acute Care Surg 2015; 79(4 Suppl 2): S227–35.
4. Mabry RL, DeLorenzo R. Challenges to improving combat casualty survival on the battlefield. Mil Med 2014; 179(5): 477–82.
5. Kelly JF, Ritenour AE, McLaughlin DF, et al. Injury severity and causes of death from Operation Iraqi Freedom and Operation Enduring Freedom: 2003–2004 versus 2006. J Trauma 2008; 64(2 Suppl): S21–6; discussion S6–7.
6. Holcomb JB, Stansbury LG, Champion HR, Wade C, Bellamy RF. Understanding combat casualty care statistics. J Trauma 2006; 60(2): 397–401.
7. Manring MM, Hawk A, Calhoun JH, Andersen RC. Treatment of war wounds: a historical review. Clin Orthop Relat Res 2009; 467(8): 2168–91.
8. Ficke JR EB, Butler FK, Alvarez J, Brown T, Pasquina P, Stoneman P, Caravalho J. Dismounted complex blast injury report of the army dismounted complex blast injury task force. J Trauma Acute Care Surg 2012; 73(6;Suppl 5): S520–34.
9. Jackson PC, Foster M, Fries A, Jeffery SL. Military trauma care in Birmingham: observational study of care requirements and resource utilisation. Injury 2014; 45(1): 44–9.
10. Eastridge BJ, Mabry RL, Seguin P, et al. Death on the battlefield (2001–2011): implications for the future of combat casualty care. J Trauma Acute Care Surg 2012; 73(6 Suppl 5): S431–7.

11. Warkentien T, Rodriguez C, Lloyd B, et al. Invasive mold infections following combat-related injuries. Clin Infect Dis 2012; 55(11): 1441–9.

12. Belmont PJ, Jr., McCriskin BJ, Sieg RN, Burks R, Schoenfeld AJ. Combat wounds in Iraq and Afghanistan from 2005 to 2009. J Trauma Acute Care Surg 2012; 73(1): 3–12.

13. Belmont PJ, Jr., Thomas D, Goodman GP, et al. Combat musculoskeletal wounds in a US Army Brigade Combat Team during operation Iraqi Freedom. J Trauma 2011; 71(1): E1–7.

14. McDonald JR, Liang SY, Li P, et al. Infectious Complications After Deployment Trauma: Following Wounded US Military Personnel Into Veterans Affairs Care. Clin Infect Dis 2018; 67(8): 1205–12.

15. Weintrob AC, Murray CK, Xu J, et al. Early Infections Complicating the Care of Combat Casualties from Iraq and Afghanistan. Surg Infect (Larchmt) 2018; 19(3): 286–97.

16. Tribble DR, Krauss MR, Murray CK, et al. Epidemiology of Trauma-Related Infections among a Combat Casualty Cohort after Initial Hospitalization: The Trauma Infectious Disease Outcomes Study. Surg Infect (Larchmt) 2018; 19(5): 494–503.

17. Murray CK, Obremskey WT, Hsu JR, et al. Prevention of infections associated with combat-related extremity injuries. J Trauma 2011; 71(2 Suppl 2): S235–57.

18. Dougherty AL, Mohrle CR, Galarneau MR, Woodruff SI, Dye JL, Quinn KH. Battlefield extremity injuries in Operation Iraqi Freedom. Injury 2009; 40(7): 772–7.

19. Eardley WG, Brown KV, Bonner TJ, Green AD, Clasper JC. Infection in conflict wounded. Philos Trans R Soc Lond B Biol Sci 2011; 366(1562): 204–18.

20. Chandler H, MacLeod K, Penn-Barwell JG, Severe Lower Extremity Combat Trauma Study G. Extremity injuries sustained by the UK military in the Iraq and Afghanistan conflicts: 2003–2014. Injury 2017; 48(7): 1439–43.

21. Pruitt BA, Jr., Rasmussen TE. Vietnam (1972) to Afghanistan (2014): the state of military trauma care and research, past to present. J Trauma Acute Care Surg 2014; 77(3 Suppl 2): S57–65.

22. Cordts PR, Brosch LA, Holcomb JB. Now and then: combat casualty care policies for Operation Iraqi Freedom and Operation Enduring Freedom compared with those of Vietnam. J Trauma **2008**; 64(2 Suppl): S14–20; discussion S.

23. Murray CK, Wilkins K, Molter NC, et al. Infections in combat casualties during Operations Iraqi and Enduring Freedom. J Trauma 2009; 66(4 Suppl): S138–44.

24. Tribble DR, Conger NG, Fraser S, et al. Infection-associated clinical outcomes in hospitalized medical evacuees after traumatic injury: trauma infectious disease outcome study. J Trauma 2011; 71(1 Suppl): S33–42.

25. Murray CK. Infectious disease complications of combat-related injuries. Crit Care Med 2008; 36(7 Suppl): S358–64.

26. Tong MJ. Septic complications of war wounds. JAMA 1972; 219(8): 1044–7.

27. Maurya S, Bhandari PS. Negative Pressure Wound Therapy in the Management of Combat Wounds: A Critical Review. Adv Wound Care (New Rochelle) 2016; 5(9): 379–89.

28. Ficke JR, Pollak AN. Extremity War Injuries: Development of Clinical Treatment Principles. J Am Acad Orthop Surg 2007; 15(10): 590–5.

29. Service JT. Tactical Combat Casualty Care Guidelines 01 Aug 2019. https://jts.amedd.army.mil/index.cfm/PI_CPGs/cpgs **2019**.

30. Lack WD, Karunakar MA, Angerame MR, et al. Type III open tibia fractures: immediate antibiotic prophylaxis minimizes infection. J Orthop Trauma 2015; 29(1): 1–6.

31. Butler FK, Jr., Blackbourne LH. Battlefield trauma care then and now: a decade of Tactical Combat Casualty Care. J Trauma Acute Care Surg 2012; 73(6 Suppl 5): S395–402.

32. Yun HC, Murray CK, Nelson KJ, Bosse MJ. Infection After Orthopaedic Trauma: Prevention and Treatment. J Orthop Trauma 2016; 30 Suppl 3: S21–S6.

33. Butler F, O'Connor K. Antibiotics in tactical combat casualty care 2002. Mil Med 2003; 168(11): 911–4.

34. Murray CK, Hospenthal DR, Kotwal RS, Butler FK. Efficacy of point-of-injury combat antimicrobials. J Trauma 2011; 71(2 Suppl 2): S307–13.

35. Mazurek MT, Ficke JR. The scope of wounds encountered in casualties from the global war on terrorism: from the battlefield to the tertiary treatment facility. J Am Acad Orthop Surg **2006**; 14(10 Spec No.): S18–23.

36. Duyos OA, Beaton-Comulada D, Davila-Parrilla A, et al. Management of Open Tibial Shaft Fractures: Does the Timing of Surgery Affect Outcomes? J Am Acad Orthop Surg 2017; 25(3): 230–8.

37. Srour M, Inaba K, Okoye O, et al. Prospective evaluation of treatment of open fractures: effect of time to irrigation and debridement. JAMA Surg 2015; 150(4): 332–6.

38. Weber D, Dulai SK, Bergman J, Buckley R, Beaupre LA. Time to initial operative treatment following open fracture does not impact development of deep infection: a prospective cohort study of 736 subjects. J Orthop Trauma 2014; 28(11): 613–9.

39. Schenker ML, Yannascoli S, Baldwin KD, Ahn J, Mehta S. Does timing to operative debridement affect infectious complications in open long-bone fractures? A systematic review. J Bone Joint Surg Am 2012; 94(12): 1057–64.

40. Prodromidis AD, Charalambous CP. The 6-Hour Rule for Surgical Debridement of Open Tibial Fractures: A Systematic Review and Meta-Analysis of Infection and Nonunion Rates. J Orthop Trauma 2016; 30(7): 397–402.

41. Murray CK, Hsu JR, Solomkin JS, et al. Prevention and management of infections associated with combat-related extremity injuries. J Trauma 2008; 64(3 Suppl): S239–51.

42. Brown KV, Guthrie HC, Ramasamy A, Kendrew JM, Clasper J. Modern military surgery: lessons from Iraq and Afghanistan. J Bone Joint Surg Br 2012; 94(4): 536–43.

43. Stinner DJ, Keeney JA, Hsu JR, et al. Outcomes of internal fixation in a combat environment. J Surg Orthop Adv 2010; 19(1): 49–53.

44. Large TM, Bonds C, Howard M. Internal fixation in a combat theater hospital. Orthopedics 2013; 36(8): 610–8.

45. Gordon W, Talbot M, Fleming M, Shero J, Potter B, Stockinger ZT. High Bilateral Amputations and Dismounted Complex Blast Injury (DCBI). Mil Med **2018**; 183(suppl_2): 118–22.

46. Gordon Wade MT, John Shero, Charles Osier, Anthony Johnson, Luke Balsamo, Zsolt Stockinger. Acute Extremity Compartment Syndrome (CS) and the role of Fasciotomy in Extremity War Wounds (CPG ID:17). Joint Trauma System Clinical Practice Guideline (JTS CPG) **2016**.

47. Blackbourne LH, Baer DG, Eastridge BJ, et al. Military medical revolution: deployed hospital and en route care. J Trauma Acute Care Surg 2012; 73(6 Suppl 5): S378–87.

48. Palm K, Apodaca A, Spencer D, et al. Evaluation of military trauma system practices related to complications after injury. J Trauma Acute Care Surg 2012; 73(6 Suppl 5): S465–71.

49. Cap AP, Pidcoke HF, Spinella P, et al. Damage Control Resuscitation. Mil Med **2018**; 183(suppl_2): 36-43.

50. Cap AP, Beckett A, Benov A, et al. Whole Blood Transfusion. Mil Med **2018**; 183(suppl_2): 44–51.

51. Hospenthal DR, Murray CK, Andersen RC, et al. Guidelines for the prevention of infections associated with combat-related injuries: 2011 update: endorsed by the Infectious Diseases Society of America and the Surgical Infection Society. J Trauma 2011; 71(2 Suppl 2): S210–34.

52. Hauser CJ, Adams CA, Jr., Eachempati SR, Council of the Surgical Infection S. Surgical Infection Society guideline: prophylactic antibiotic use in open fractures: an evidence-based guideline. Surg Infect (Larchmt) 2006; 7(4): 379–405.

53. Hoff WS, Bonadies JA, Cachecho R, Dorlac WC. East Practice Management Guidelines Work Group: update to practice management guidelines for prophylactic antibiotic use in open fractures. J Trauma 2011; 70(3): 751–4.

54. Bennett PM, Sargeant ID, Myatt RW, Penn-Barwell JG. The management and outcome of open fractures of the femur sustained on the battlefield over a ten-year period. The bone & joint journal **2015**; 97-b(6): 842–6.

55. Mack AW, Freedman BA, Groth AT, Kirk KL, Keeling JJ, Andersen RC. Treatment of open proximal femoral fractures sustained in combat. J Bone Joint Surg Am 2013; 95(3): e13(1–8).

56. Saeed O, Tribble DR, Biever KA, Crouch HK, Kavanaugh M. Infection Prevention in Combat-Related Injuries. Mil Med 2018; 183(suppl_2): 137–41.

57. Tribble DR, Lloyd B, Weintrob A, et al. Antimicrobial prescribing practices following publication of guidelines for the prevention of infections associated with combat-related injuries. J Trauma 2011; 71(2 Suppl 2): S299–306.

58. Lalliss SJ, Stinner DJ, Waterman SM, Branstetter JG, Masini BD, Wenke JC. Negative pressure wound therapy reduces pseudomonas wound contamination more than Staphylococcus aureus. J Orthop Trauma 2010; 24(9): 598–602.

59. Geiger S, McCormick F, Chou R, Wandel AG. War wounds: lessons learned from Operation Iraqi Freedom. Plast Reconstr Surg 2008; 122(1): 146–53.

60. Warner M, Henderson C, Kadrmas W, Mitchell DT. Comparison of vacuum-assisted closure to the antibiotic bead pouch for the treatment of blast injury of the extremity. Orthopedics 2010; 33(2): 77–82.

61. Hinck D, Franke A, Gatzka F. Use of vacuum-assisted closure negative pressure wound therapy in combat-related injuries – literature review. Mil Med 2010; 175(3): 173–81.

62. Leininger BE, Rasmussen TE, Smith DL, Jenkins DH, Coppola C. Experience with wound VAC and delayed primary closure of contaminated soft tissue injuries in Iraq. J Trauma 2006; 61(5): 1207–11.

63. Davis KA, Moran KA, McAllister CK, Gray PJ. Multidrug-resistant Acinetobacter extremity infections in soldiers. Emerg Infect Dis 2005; 11(8): 1218–24.

64. Centers for Disease C, Prevention. Acinetobacter baumannii infections among patients at military medical facilities treating injured U.S. service members, 2002–2004. MMWR Morb Mortal Wkly Rep 2004; 53(45): 1063–6.

65. Scott P, Deye G, Srinivasan A, et al. An outbreak of multidrug-resistant Acinetobacter baumannii-calcoaceticus complex infection in the US military health care system associated with military operations in Iraq. Clin Infect Dis 2007; 44(12): 1577–84.

66. Sebeny PJ, Riddle MS, Petersen K. Acinetobacter baumannii skin and soft-tissue infection associated with war trauma. Clin Infect Dis 2008; 47(4): 444–9.

67. Griffith ME, Ceremuga JM, Ellis MW, Guymon CH, Hospenthal DR, Murray CK. Acinetobacter skin colonization of US Army Soldiers. Infect Control Hosp Epidemiol 2006; 27(7): 659–61.

68. Griffith ME, Ellis MW, Murray CK. Acinetobacter nares colonization of healthy US soldiers. Infect Control Hosp Epidemiol 2006; 27(7): 787–8.

69. Griffith ME, Gonzalez RS, Holcomb JB, Hospenthal DR, Wortmann GW, Murray CK. Factors associated with recovery of Acinetobacter baumannii in a combat support hospital. Infect Control Hosp Epidemiol 2008; 29(7): 664–6.

70. Yun HC, Murray CK, Roop SA, Hospenthal DR, Gourdine E, Dooley DP. Bacteria recovered from patients admitted to a deployed U.S. military hospital in Baghdad, Iraq. Mil Med 2006; 171(9): 821–5.

71. Petersen K, Riddle MS, Danko JR, et al. Trauma-related infections in battlefield casualties from Iraq. Ann Surg 2007; 245(5): 803-11.

72. Murray CK, Hinkle MK, Yun HC. History of infections associated with combat-related injuries. J Trauma 2008; 64(3 Suppl): S221–31.

73. Schreiber MA, Zink K, Underwood S, Sullenberger L, Kelly M, Holcomb JB. A comparison between patients treated at a combat support hospital in Iraq and a Level I trauma center in the United States. J Trauma 2008; 64(2 Suppl): S118–21; discussion S21–2.

74. Yun HC, Blackbourne LH, Jones JA, et al. Infectious complications of noncombat trauma patients provided care at a military trauma center. Mil Med 2010; 175(5): 317–23.

75. Stewart L, Shaikh F, Bradley W, et al. Combat-Related Extremity Wounds: Injury Factors Predicting Early Onset Infections. Mil Med 2019; 184(Suppl 1): 83–91.

76. Tribble DR, Li P, Warkentien TE, et al. Impact of Operational Theater on Combat and Noncombat Trauma-Related Infections. Mil Med 2016; 181(10): 1258–68.

77. Penn-Barwell JG, Bennett PM, Mortiboy DE, Fries CA, Groom AF, Sargeant ID. Factors influencing infection in 10 years of battlefield open tibia fractures. Strategies Trauma Limb Reconstr 2016; 11(1): 13–8.

78. Brown KV, Murray CK, Clasper JC. Infectious complications of combat-related mangled extremity injuries in the British military. J Trauma 2010; 69 Suppl 1: S109–15.

79. Murray CK, Roop SA, Hospenthal DR, et al. Bacteriology of war wounds at the time of injury. Mil Med 2006; 171(9): 826–9.

80. Wallum TE, Yun HC, Rini EA, et al. Pathogens present in acute mangled extremities from Afghanistan and subsequent pathogen recovery. Mil Med 2015; 180(1): 97–103.

81. Hospenthal DR, Crouch HK, English JF, et al. Multidrug-resistant bacterial colonization of combat-injured personnel at admission to medical centers after evacuation from Afghanistan and Iraq. J Trauma 2011; 71(1 Suppl): S52–7.

82. Mende K, Beckius ML, Zera WC, et al. Phenotypic and genotypic changes over time and across facilities of serial colonizing and infecting Escherichia coli isolates recovered from injured service members. J Clin Microbiol 2014; 52(11): 3869–77.

83. Weintrob AC, Murray CK, Lloyd B, et al. Active surveillance for asymptomatic colonization with multidrug-resistant gram negative bacilli among injured service members – a three year evaluation. MSMR 2013; 20(8): 17–22.

84. Keen EF, 3rd, Murray CK, Robinson BJ, Hospenthal DR, Co EM, Aldous WK. Changes in the incidences of multidrug-resistant and extensively drug-resistant organisms isolated in a military medical center. Infect Control Hosp Epidemiol 2010; 31(7): 728–32.

85. Campbell WR, Li P, Whitman TJ, et al. Multi-Drug-Resistant Gram-Negative Infections in Deployment-Related Trauma Patients. Surg Infect (Larchmt) 2017; 18(3): 357–67.

86. Mende K, Stewart L, Shaikh F, et al. Microbiology of combat-related extremity wounds: Trauma Infectious Disease Outcomes Study. Diagn Microbiol Infect Dis 2019; 94(2): 173–9.

87. Alga A, Wong S, Shoaib M, et al. Infection with high proportion of multidrug-resistant bacteria in conflict-related injuries is associated with poor outcomes and excess resource consumption: a cohort study of Syrian patients treated in Jordan. BMC Infect Dis 2018; 18(1): 233.

88. Akers KS, Mende K, Cheatle KA, et al. Biofilms and persistent wound infections in United States military trauma patients: a case-control analysis. BMC Infect Dis 2014; 14: 190.

89. Burns TC, Stinner DJ, Mack AW, et al. Microbiology and injury characteristics in severe open tibia fractures from combat. J Trauma Acute Care Surg 2012; 72(4): 1062–7.

90. Doucet JJ, Galarneau MR, Potenza BM, et al. Combat versus civilian open tibia fractures: the effect of blast mechanism on limb salvage. J Trauma 2011; 70(5): 1241–7.

91. Tribble DR, Lewandowski LR, Potter BK, et al. Osteomyelitis Risk Factors Related to Combat Trauma Open Tibia Fractures: A Case-Control Analysis. J Orthop Trauma 2018; 32(9): e344–e53.

92. Yun HC, Branstetter JG, Murray CK. Osteomyelitis in military personnel wounded in Iraq and Afghanistan. J Trauma **2008**; 64(2 Suppl): S163–8; discussion S8.

93. Mody RM, Zapor M, Hartzell JD, et al. Infectious complications of damage control orthopedics in war trauma. J Trauma 2009; 67(4): 758–61.

94. Lewandowski LR, Potter BK, Murray CK, et al. Osteomyelitis Risk Factors Related to Combat Trauma Open Femur Fractures: A Case-Control Analysis. J Orthop Trauma 2019; 33(4): e110–e9.

95. Johnson EN, Burns TC, Hayda RA, Hospenthal DR, Murray CK. Infectious complications of open type III tibial fractures among combat casualties. Clin Infect Dis 2007; 45(4): 409–15.

96. Osmon DR, Berbari EF, Berendt AR, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2013; 56(1): e1–e25.

97. Della Valle C, Parvizi J, Bauer TW, et al. American Academy of Orthopaedic Surgeons clinical practice guideline on: the diagnosis of periprosthetic joint infections of the hip and knee. J Bone Joint Surg Am 2011; 93(14): 1355–7.

98. Napierala MA, Rivera JC, Burns TC, et al. Infection reduces return-to-duty rates for soldiers with Type III open tibia fractures. J Trauma Acute Care Surg 2014; 77(3 Suppl 2): S194–7.

99. Li HK, Scarborough M, Zambellas R, et al. Oral versus intravenous antibiotic treatment for bone and joint infections (OVIVA): study protocol for a randomised controlled trial. Trials 2015; 16: 583.

100. Lloyd B, Weintrob AC, Rodriguez C, et al. Effect of early screening for invasive fungal infections in U.S. service members with explosive blast injuries. Surg Infect (Larchmt) **2014**; 15(5): 619–26.

101. Murray CK, Gross K, Russell RJ, Haslett RA. Dismounted Complex Blast Injuries Including Invasive Fungal Infections. US Army Med Dep J **2016**; (2–16): 24–8.

102. Rodriguez CJ, Weintrob AC, Shah J, et al. Risk factors associated with invasive fungal infections in combat trauma. Surg Infect (Larchmt) 2014; 15(5): 521–6.

103. Tribble DR, Rodriguez CJ. Combat-Related Invasive Fungal Wound Infections. Curr Fungal Infect Rep 2014; 8(4): 277–86.

104. Weintrob AC, Weisbrod AB, Dunne JR, et al. Combat trauma-associated invasive fungal wound infections: epidemiology and clinical classification. Epidemiol Infect 2015; 143(1): 214–24.

105. Rodriguez CJ, Tribble DR, Malone DL, et al. Treatment of Suspected Invasive Fungal Infection in War Wounds. Mil Med **2018**; 183(suppl_2): 142–6.

106. Ganesan A, Shaikh F, Bradley W, et al. Classification of Trauma-Associated Invasive Fungal Infections to Support Wound Treatment Decisions. Emerg Infect Dis 2019; 25(9).

107. Warkentien TE, Shaikh F, Weintrob AC, et al. Impact of Mucorales and Other Invasive Molds on Clinical Outcomes of Polymicrobial Traumatic Wound Infections. J Clin Microbiol 2015; 53(7): 2262–70.

108. Blyth DM, Mende K, Weintrob AC, et al. Resistance patterns and clinical significance of Candida colonization and infection in combat-related injured patients from Iraq and Afghanistan. Open Forum Infect Dis **2014**; 1(3): ofu109.

109. Neblett Fanfair R, Benedict K, Bos J, et al. Necrotizing cutaneous mucormycosis after a tornado in Joplin, Missouri, in 2011. N Engl J Med 2012; 367(23): 2214–25.

110. Weddle G, Pahud B, Jackson MA. Mucormycosis after a tornado in Joplin, Missouri. N Engl J Med 2013; 368(11): 1066–7.

111. Rodriguez C, Weintrob AC, Dunne JR, et al. Clinical relevance of mold culture positivity with and without recurrent wound necrosis following combat-related injuries. J Trauma Acute Care Surg 2014; 77(5): 769–73.

112. Lewandowski LR, Weintrob AC, Tribble DR, et al. Early Complications and Outcomes in Combat Injury-Related Invasive Fungal Wound Infections: A Case-Control Analysis. J Orthop Trauma 2016; 30(3): e93–9.

113. Tribble DR, Rodriguez CJ, Weintrob AC, et al. Environmental Factors Related to Fungal Wound Contamination after Combat Trauma in Afghanistan, 2009–2011. Emerg Infect Dis 2015; 21(10): 1759–69.

114. Waterman P, Kwak Y, Clifford R, et al. A multidrug-resistance surveillance network: 1 year on. Lancet Infect Dis 2012; 12(8): 587–8.

115. Co EM, Aldous WK, Keen E, 3rd, Robinson B, Hamilton LR. Improving detection of extended-spectrum beta-lactamase-producing bacteria in a deployed setting. US Army Med Dep J 2011: 70–3.

116. Lesho E, Lin X, Clifford R, et al. From the Battlefield to the Bedside: Supporting Warfighter and Civilian Health With the "ART" of Whole Genome Sequencing for Antibiotic Resistance and Outbreak Investigations. Mil Med 2016; 181(7): 621–4.

117. Frickmann H, Podbielski A, Kreikemeyer B. Resistant Gram-Negative Bacteria and Diagnostic Point-of-Care Options for the Field Setting during Military Operations. Biomed Res Int 2018; 2018: 9395420.
118. Be NA, Allen JE, Brown TS, et al. Microbial profiling of combat wound infection through detection microarray and next-generation sequencing. J Clin Microbiol 2014; 52(7): 2583–94.
119. Ganesan A, Wells J, Shaikh F, et al. Molecular Detection of Filamentous Fungi in Formalin-Fixed Paraffin-Embedded Specimens in Invasive Fungal Wound Infections is Feasible with High Specificity. J Clin Microbiol 2019.
120. Fares Y, El-Zaatari M, Fares J, Bedrosian N, Yared N. Trauma-related infections due to cluster munitions. J Infect Public Health 2013; 6(6): 482–6.
121. Wolf DG, Polacheck I, Block C, et al. High rate of candidemia in patients sustaining injuries in a bomb blast at a marketplace: a possible environmental source. Clin Infect Dis 2000; 31(3): 712–6.
122. Alga A, Karlow Herzog K, Alrawashdeh M, Wong S, Khankeh H, Stalsby Lundborg C. "Reality rarely looks like the guidelines": a qualitative study of the challenges hospital-based physicians encounter in war wound management. Scand J Trauma Resusc Emerg Med **2018**; 26(1): 52.
123. Kellermann AL, Peleg K. Lessons from Boston. N Engl J Med 2013; 368(21): 1956–7.

Index