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- What's New in Musculoskeletal Infections?
- What Is the Most Effective Treatment for Periprosthetic Joint Infection After Total Joint Arthroplasty in Patients with Rheumatoid Arthritis?
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Guest Editorial
What's New in Musculoskeletal Infection

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Musculoskeletal infection continues to be the most devastating complication after orthopaedic surgery. It is a burden for all involved: painful for patients, challenging for physicians, and costly for the health-care system. A tremendous amount of research has been devoted in 2022 to understanding and solving many problems associated with musculoskeletal infection. The intent of this article is to highlight key studies that augment current knowledge regarding prevention, diagnosis, and treatment. Although the majority of infection research has been in the field of hip and knee arthroplasty, this article will also cover landmark studies in other subspecialties from the past year.

A substantial body of research was dedicated to the psychosocial effect of periprosthetic joint infection (PJI) on patients. Furdock et al. showed that 20% of patients who underwent 2-stage exchange for PJI presented with Patient-Reported Outcomes Measurement Information System (PROMIS) depression scores consistent with major depressive disorder, compared with 7% of patients who underwent aseptic revision. After treatment, depression scores improved in both cohorts[1]. In a study utilizing the PearlDiver Database, Das et al. reported that the risk of depressive, anxiety, bipolar, psychotic, and stress disorders was significantly higher in patients who underwent spacer placement for PJI than in patients who underwent aseptic revision[2]. In another study, Lueck et al.[3] demonstrated that patients who undergo spacer insertion for PJI experience a significant decline in psychological health as determined by the 36-Item Short Form Health Survey[4] (SF-36) and Hospital Anxiety and Depression Scale[5] (HADS). Similar findings were also reported in the Girdlestone population using PROMIS Global Physical and Mental Health surveys[6]. It is not surprising that treatment regret with respect to having undergone primary total joint arthroplasty (TJA) is a phenomenon experienced by 28% of patients who underwent hip and knee arthroplasty and experienced PJI that required 2-stage exchange, as discussed by Sequeira et al.[7].

Prevention

Although we still search for ways to improve our treatment outcomes for PJI, most surgeons would agree that prevention is the most important step in the management of this very difficult problem. Several recent studies have tried to identify the optimal irrigation solution to prevent PJI in primary total hip arthroplasty (THA) and total knee arthroplasty (TKA). A retrospective review of >30,000 cases demonstrated a reduction in PJI rates with the use of dilute povidone-iodine solution[8]. Another basic science study found that povidone-iodine, sodium hypochlorite, and acetic acid-based irrigants all demonstrated eradication of all bacterial growth in <2 minutes of contact[9]. Five of 7 trials in a recent systematic review and meta-analysis found a benefit in reducing PJI rates with the use of topical vancomycin powder and povidone-iodine solution, but the studies were of poorer quality with varying dosing and also found higher rates of wound complications in those patients receiving vancomycin powder alone[10]. A systematic review and meta-analysis published last year specifically on the use of vancomycin powder did find a reduction in PJI rates; however, the quality of those studies were poor as well[11]. The optimal irrigation solution, which should balance bactericidal activity with lack of inhibition of wound-healing, still has not been conclusively determined. Further prospective randomized clinical trials are needed to answer this important question.

Similarly, much research has focused on the optimal dressing to prevent PJI, especially in high-risk patients. Although negative-pressure wound therapy and silver-impregnated dressings both have data supporting their use, a recent randomized controlled trial found no difference between the 2 dressings in obese patients[12]. Over the last few years, orthopaedic surgeons have made great progress in optimizing modifiable risk factors prior to arthroplasty, specifically with weight loss before the surgical procedure. With more patients undergoing bariatric surgery to optimize their weight, a recent study found that patients who underwent bariatric surgery actually had higher rates of reoperation for PJI after TKA relative to a matched cohort with high body mass index (BMI), suggesting that underlying malnutrition may play a role[13]. Likewise, patients who underwent bariatric surgery prior to THA had higher rates of implant failure and dislocation than patients with naturally low or high BMI[14].

Other perioperative protocols continue to be evaluated to reduce the risk of PJI. A prospective cohort study in >1,200 patients who underwent primary TKA found that those who wiped the surgical area with chlorhexidine the night before the surgical procedure had lower infection rates[15]. The optimal venous thromboembolic prophylaxis continues to be debated.

Disclosure: The **Disclosure of Potential Conflicts of Interest** forms are provided with the online version of the article (http://links.lww.com/JBJS/H523).

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A recent study found that patients taking lower-dose aspirin (81 mg twice daily) had lower rates of PJI than patients taking a higher dose (325 mg twice daily)[16].

Intraoperatively, many surgeons prefer the use of helmets, but concerns exist with regard to the potential contamination of the fan. One study suggested that the fan should run for 3 minutes prior to entering the operating room to decrease the risk of contamination[17]. We have made great strides reducing the rates of PJI following primary TKA and THA over the last decade, but there is more work to be done. Further prospective research on prevention of PJI should focus on continuing to improve our patient optimization and perioperative protocols.

Diagnosis

Advances in the diagnosis of PJI involved several key areas: clinical testing of novel serum and synovial fluid laboratory markers, the predictive value of testing prior to reimplantation in chronic PJI, and the exploration of diagnostic imaging modalities.

In a single-institution study of 7,661 patients, Aichmair et al. assessed the predictive value of serum interleukin (IL)-6, which has a shorter half-life than C-reactive protein (CRP), in early-onset PJI after THA and TKA[18]. IL-6 levels measured on postoperative day 3 demonstrated no significant difference in patients who underwent THA or TKA with and without early-onset PJI. In a retrospective case-control study, Yan et al. investigated superoxide dismutase (SOD) as a potential novel serum biomarker in the diagnosis of PJI after TKA[19]. The authors concluded that serum SOD represents a promising marker, including in a subgroup analysis in culture-negative PJI.

In a prospective study of synovial pH, Theil et al. compared this value with other traditional markers of chronic PJI after THA and TKA[20]. Synovial pH was found to be a useful adjunct parameter to established synovial markers such as synovial leukocyte count and differential, but showed low sensitivity. Grzelecki et al. sought to determine the utility of a rapid, off-label strip test that detects D-lactic acid in synovial fluid in the diagnosis of PJI[21]. In their prospective study of revision THA and TKA, the authors found good accuracy, with comparable sensitivity and specificity to leukocyte esterase (LE) strip tests. Another study examined the proteomic profiling of sonicated fluid to further support this potential avenue to differentiate PJI from noninfectious arthroplasty failure[22]. A study of serum and synovial markers of early PJI found that false-negative rates were significantly higher for synovial white blood-cell counts and synovial neutrophil percentage in patients treated with antibiotics within 2 weeks compared with untreated patients[23].

With respect to reimplantation arthroplasty algorithms, Shao et al. evaluated the diagnostic effectiveness of serum CRP, erythrocyte sedimentation rate (ESR), plasma D-dimer, and fibrinogen obtained prior to performing second-stage revision or spacer exchange[24]. The authors reported that plasma fibrinogen had the highest area under the receiving operating characteristic curve (AUC) value of 0.831, followed by serum CRP (0.829) and ESR (0.795); plasma D-dimer had the lowest AUC value of 0.716. The authors of another study concluded that routine use of alpha-defensin in the workup prior to a second-stage arthroplasty for PJI may not be warranted[25].

In a retrospective study of triple-phase bone scanning in the setting of potential PJI, semiquantitative criteria showed no advantage in PJI diagnosis[26]. The authors observed no significant difference between visual analysis and semiquantitative measurement in terms of sensitivity, specificity, positive predictive value, negative predictive value, and accuracy. Triple-phase bone scanning demonstrated good clinical diagnostic efficacy when the time interval from prosthesis implantation to bone scanning was >1 year.

Surgical Treatment

Published research in the past year continues to clarify the role of each of the 3 major treatment options for PJI: irrigation and debridement, 2-stage exchange, and 1-stage exchange.

Irrigation and Debridement

The timing of debridement, antibiotics, and implant retention (DAIR) continues to show importance in the literature. A comparison study between DAIR and 2-stage revision within 12 weeks of the index arthroplasty showed comparable success rates of each technique at the 6-year follow-up, supporting the importance of timing with regard to performing DAIR[27]. DAIR continues to appear to be an acceptable treatment in management of early PJI (within 30 days) after revision arthroplasty; however, failure rates are increased in cases of antibiotic mismatches, multiple DAIR procedures, or a prolonged interval (>30 days) from the index procedure to the DAIR[28]. The addition of antibiotic-loaded calcium sulfate beads has not been shown to reduce the incidence of recurrent PJIs following DAIR[29]. A registry-based cohort study showed no difference in re-revision rates of an initial 2-stage exchange compared with a 2-stage exchange following a failed DAIR[30].

Two-Stage Exchange

The results of 2-stage exchange continue to show improved success rates when compared with DAIR for chronic knee PJI. A multicenter study with a minimum 5-year follow-up of PJI in knees showed an infection eradication rate of 89%. High mortality, 33% in 1 study, continues to be seen during the course of 2-stage treatment[31]. The eradication rates of PJI in knees were similar to those seen in PJI in hips[32]. The risk factors for reinfection following 2-stage exchange for PJI were elevated CRP levels at the time of diagnosis and infection with methicillin-sensitive *Staphylococcus aureus* (MSSA)[33]. The use of a short course of oral antibiotics (<2 weeks) has been shown to decrease the 1-year reinfection rate following 2-stage exchange arthroplasty for PJI[34].

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Failed 2-stage exchange remains a large financial burden on the health-care system. Patients undergoing successful 2-stage exchange for hip PJI without a further surgical procedure incurred approximately $40,000 less total costs than those requiring further surgical procedures following reimplantation[35]. High-dose antibiotic cement spacers for the treatment of PJI were found to be independent risk factors for acute kidney injury, which had a rate of 22.7% following the first stage of a planned 2-stage exchange versus 6.6% following a 1-stage exchange[36]. A study comparing knee spacer types (new femoral component, cement-on-cement, and static) found no difference in the odds of infection clearance and showed increased range of motion and improved ambulatory status prior to reimplantation utilizing the new-femoral-component spacer design[37].

Although downtrending serum markers can be reassuring prior to reimplantation, there do not appear to be values for ESR or CRP that significantly predict failed 2-stage exchange for PJI; thus, pre-reimplantation aspiration is recommended to help to guide management[38].

One-Stage Exchange

Although a prospective, multicenter, randomized study comparing 1-stage exchange with 2-stage exchange is ongoing in the United States, the results are not yet available. However, 1-stage exchange continues to gain enthusiasm as a treatment for PJI despite varied results. One study showed a re-revision rate for infection of 20% at 8 years in 1-stage exchange for streptococcal hip PJI[39]. The design of constructs used in 1-stage exchange for PJI also appear to vary among institutions. A study comparing 1-stage exchange utilizing a metal femoral component and an all-polyethylene tibial component compared with 2-stage exchange showed improved infection-free survival at 2 years (85% compared with 75%) and overall lower postoperative complication rates[40]. Implant design (hinged compared with non-hinged TKA) in 1-stage exchange did not show significantly different functional outcomes across cohorts, and the designs showed an overall infection control rate of 91% at a mean follow-up of 6 years[41].

Antibiotic Therapy

Antibiotic Prophylaxis

A prospective, multicenter study of 1,838 patients who underwent primary TJA demonstrated that a weight-adjusted preoperative dose of cephalosporin was associated with lower surgical site infection risk compared with alternative antibiotics administered at or after the time of incision[42]. Prophylactic antibiotic administration for >24 hours was not associated with a decreased risk of surgical site infection.

In patients who underwent TJA and were at high risk for PJI, extended oral antibiotic prophylaxis for 7 days with cefadroxil, trimethoprim-sulfamethoxazole, or clindamycin was found to be a cost-effective measure to decrease the rate of PJI[43].

Two studies concluded that extended oral antibiotic prophylaxis (for 7 days in 1 study and a mean of 11 days in the other) with cefadroxil or cephalexin after aseptic revision TKA results in a significantly lower rate of PJI at 90 days[44,45]. However, the same extended postoperative antibiotic regimen as in the latter study (mean, 11 days) after aseptic revision THA did not confer any decreased risk of PJI[46].

Antibiotics and PJI

Based on a multicenter study evaluating the species and antibiotic resistance profiles of infecting organisms in PJI after TKA, the most effective empiric antibiotic regimen once culture results have been obtained is vancomycin for infections that occur <1 year after the surgical procedure and cefazolin for infections that occur later[47].

A prospective, randomized controlled trial demonstrated that the use of an antibiotic spacer with 2 g of vancomycin and 2.4 g of tobramycin per bag of PALACOS cement (Heraeus Medical) in the treatment of PJI is an independent risk factor for acute kidney injury, particularly in patients with chronic kidney disease[36].

In patients who underwent failed surgical treatment for PJI, chronic oral antibiotic suppression yielded 67% reoperation-free survival at a median follow-up of 50 months[48]. Patients with THA or gram-positive infections had increased likelihood of success with suppressive antibiotic therapy. Another approach following multiple failed surgical treatments for PJI is 1-stage revision with intra-articular antibiotic infusion, reported to have an 87.6% rate of survival free from reoperation for infection at a 7-year follow-up[49].

A multicenter study found that, in patients who met the definition of culture-negative PJI but had no histologic signs of infection, antibiotic therapy could be withheld without infection recurrence at the 2-year follow-up[50].

Antibiotic Resistance

In an international, multicenter study of 218 patients, the use of gentamycin-loaded bone cement in primary TJA did not increase the prevalence of resistance to gentamycin or other antibiotics among infecting organisms in patients who developed PJI[51].

Conversely, in patients who received ≥2 weeks of oral antibiotics following reimplantation in 2-stage revision for PJI, there was increased resistance to the oral antibiotic among the infecting organisms causing recurrent PJI[52]. However, as novel resistant organisms causing reinfection were not recorded as the same species as the original infecting organism in this study cohort, it is difficult to conclude that selective pressure from oral antibiotics induced new drug resistance.

The antimicrobial resistance profile of coagulase-negative staphylococci isolated from cases of PJI after TKA was found to differ significantly between tertiary referral centers, even ones in geographic proximity to one another[53]. As a result, continuous antibiotic susceptibility testing is essential to optimize antibiotic therapy and stewardship.

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Other Topics

Animal and In Vitro PJI Models

Ibrahim et al. presented data showing reproducible results with an ingrowth hip hemiarthroplasty model for gram-negative PJI in rats. The model allows weight-bearing, shows predictable biofilm formation, and provides a clinically relevant animal model for challenging PJI cases[54]. Visperas et al. presented a novel rabbit model for knee PJI with consistent biofilm production and reproducible response to sham compared with antibiotic treatments[55]. Small animal models of musculoskeletal infection often require general anesthetic, and hypothermia in murine models is common during general anesthesia. Constant et al. demonstrated that peri-anesthetic hypothermia in rodents creates a significant risk of both greater infection burden and mortality in these models, complicating the interpretation of results across all small animal studies examining infection and outcomes[56].

In an effort to understand the precise timing and biology of *Pseudomonas aeruginosa* biofilm production, Spake et al. reported on a polyetheretherketone (PEEK) disc model for in vitro biofilm creation[57]. Their model allowed for consistent imaging and quantification of biofilm production and has implications for understanding the variables associated with biofilm production across multiple species.

Genetics, Genomics, and Novel Therapeutics

The sequencing of pathogens to understand the individual genotype has started to become relevant to both research and clinical treatment of orthopaedic infections in the past few years. Trobos et al. presented data from a unique study that attempted to correlate genomic bacterial data with patient outcomes in PJI. They analyzed 111 staphylococcal strains obtained from patients during surgical treatment of PJI and correlated genomic data with a binary infection-treatment outcome (infection was resolved or unresolved). *Staphylococcus epidermidis* ST2 caused the majority of relapses and was associated with both multidrug resistance and strong biofilm production. Similarly, the *S. aureus* strains with the strongest biofilm production were the most likely to cause unresolved infection[58]. Small-colony variants in *S. aureus* are present in varying degrees and can predict the likelihood of invasion into osteoblasts in an in vitro model of bacterial isolates obtained from patients with diagnosed PJI, potentially helping to identify those at risk for persistent infection[59].

On the host side, CCR2 (C-C motif chemokine receptor 2) mediates chemotaxis for macrophages and neutrophils during inflammatory responses. In a murine model of orthopaedic implant-associated infection, CCR2-deficient mice were found to have significantly reduced myeloid inflammatory cells in draining lymph nodes compared with the control wild-type mice[60]. In a study evaluating the ability of orthopaedic infections to co-opt our own immune regulatory system for survival benefits, as malignancies also often do, Warren et al. analyzed periprosthetic tissue from patients undergoing revision hip or knee arthroplasty for immune checkpoints related to apoptosis (PD-1 [programmed cell death-1] and its ligand PD-L1). Patients were separated into those with aseptic diagnoses (16 patients) and those with PJI (15 patients), and were further evaluated on the basis of recurrence of infection. PD-L1 expression was upregulated ($p = 0.039$) in PJI cases (25%) compared with aseptic cases (8%), and it was upregulated ($p = 0.039$) in the recurrent PJI cases (68%) compared with the remaining PJI cases (15%). Those in whom expression of PD-L1 was >20% had an odds ratio of 15 for reinfection compared with controls ($p = 0.092$). Although the numbers are small, the series suggests immune checkpoint upregulation as a potential mechanism for recurrent or persistent orthopaedic infection[61].

In a mouse model of femoral osteomyelitis, Kobayashi et al. tested zoledronic acid and anti-RANKL (receptor activator of nuclear factor kappa-B ligand) monoclonal antibody to assess osteoprotective effects against the erosive and necrotic changes of the untreated infection. The anti-RANKL monoclonal antibody outperformed zoledronic acid and showed some promise in preventing further osteonecrosis associated with osteomyelitis[62].

Carbon-infiltrated carbon nanotube (CICNT) surfaces mimic antimicrobial surface textures found in nature and have been shown previously to have a minimal effect on osseointegration. Morco et al. performed an in vitro study of 2 different CICNT types in a biofilm model, showing that both stainless steel substrate and carbon substrate CICNTs were able to reduce biofilm burden by 60% to 80% ($p < 0.0001$) compared with controls. Applications abound for future orthopaedic implant coatings[63].

Bacteriophages

DePalma et al. described a series of staphylococcal isolates from patients with PJI and their response to available bacteriophages. They found that small-colony variants were present in 24% of the isolates and that none of these isolates had growth inhibition by the bacteriophages[64]. Totten and Patel reported on bacteriophage activity against 122 clinical isolates of *S. aureus* from patients with orthopaedic implant infections, finding successful bacteriophage infection in 73% of the planktonic bacteria and 100% of the biofilm bacteria[65]. Šuster and Cör assessed and compared bacteriophage K DNA methods for identifying staphylococcal infections with high sensitivity and specificity in a relatively short 3 to 4-hour time frame that could dramatically shorten the diagnosis for patients with orthopaedic infections[66].

Sports and Biomechanics

Sorensen et al. presented biomechanical data on tensile strength of tendon grafts affected by varying *S. epidermidis* infectious bioburden and found that infection led to a significantly decreased peak load to failure for the tendon grafts compared with controls ($p = 0.043$). The increasing burden led to an even lower peak to failure ($p = 0.0005$ at 10,000 colony-forming units)[67].

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Further adding to the data supporting vancomycin use in anterior cruciate ligament (ACL) reconstruction, Tong et al.[68] presented in vivo data from a rat model supporting specific times and concentrations for vancomycin soaking of the ACL graft. Truong et al.[69] presented findings that vancomycin-soaked grafts are highly cost-effective for ACL reconstruction.

Trauma and Infection

To understand bacterial associations with polymicrobial infection, Gitajn et al.[70] retrospectively reviewed >400 fracture-associated deep infections that required operative debridement. They found that methicillin-resistant *S. aureus* (MRSA), MSSA, and coagulase-negative staphylococcal species represented the majority of monomicrobial infections (71%). Gram-negative rods, gram-positive rods, and anaerobes were much more likely to be found in polymicrobial infections. Specific organisms from the Enterobacter, Enterococcus, and Pseudomonas genera were found to have the highest frequency in polymicrobial infections[70]. For necrotizing soft-tissue infections, Heath et al. found that early administration of clindamycin as part of the antibiotic regimen conferred a substantial limb-salvage benefit after controlling for multiple other factors[71].

Spine

Vicente-Sánchez et al. presented compelling data showing a significant decrease in the incidence of early surgical site infections in spine surgery following the implementation of surgical care bundles in 2012 (4.2% compared with 1.9%; $p = 0.006$)[72]. Karamian et al. used a retrospective 3-to-1 case-control matched study to evaluate the effect of early surgical site infections on patients after thoracolumbar fusion. Although the surgical site infection group had a higher rate of early readmission and reoperation, both groups had similar improvements in patient-reported outcomes with no differences at 1 year, suggesting that, if appropriately managed, surgical site infection after spine surgery does not lead to prolonged disability or worse clinical outcomes[73].

Foot and Ankle

Conti et al. reported on a series of 11 patients undergoing 2-stage revision total ankle arthroplasty for chronic PJI, showing a 63% reoperation rate after reimplantation and 1 below-the-knee amputation to control infection, but a majority of patients who were ambulatory at the final follow-up[74]. Winkler et al. retrospectively reviewed 583 amputations for diabetic foot osteomyelitis to determine the relation of limb loss to lesion location and other comorbidities, finding that patients with more proximal lesions and those with substantial peripheral vascular disease had a significantly higher chance of major amputation above the ankle joint[75].

Evidence-Based Orthopaedics

The editorial staff of *JBJS* reviewed a large number of recently published studies related to the musculoskeletal system that received a higher Level of Evidence grade. In addition to articles cited already in this update, 4 other articles relevant to infection are appended to this review after the standard bibliography, with a brief commentary about each article to help guide your further reading, in an evidence-based fashion, in this subspecialty area.

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Evidence-Based Orthopaedics

Blom AW, Lenguerrand E, Strange S, Noble SM, Beswick AD, Burston A, Garfield K, Gooberman-Hill R, Harris SRS, Kunutsor SK, Lane JA, MacGowan A, Mehendale S, Moore AJ, Rolfson O, Webb JCJ, Wilson M, Whitehouse MR; INFORM trial group. Clinical and cost effectiveness of single stage compared with 2 stage revision for hip prosthetic joint infection (INFORM): pragmatic, parallel group, open label, randomised controlled trial. *BMJ*. 2022 Oct 31;379:e071281.

In a prospective, randomized controlled trial, Blom et al. evaluated 140 patients with PJI of the hip and compared 1-stage with 2-stage exchange arthroplasty. There was no difference in the presumed infection eradication rate between the groups, but patients who underwent 1-stage exchange had fewer complications (8% compared with 27%; p = 0.01). Additionally, 1-stage exchange was also more cost-effective.

According to this study, surgeons should consider 1-stage exchange arthroplasty for candidate patients with PJI in order to minimize complication rates and cost of the treatment.

Kruse CC, Ekhtiari S, Oral I, Selznick A, Mundi R, Chaudhry H, Pincus D, Wolfstadt J, Kandel CE. The use of rifampin in total joint arthroplasty: a systematic review and meta-analysis of comparative studies. *J Arthroplasty*. 2022 Aug;37(8):1650-7.

In a systematic review and meta-analysis that included 22 studies analyzing the effect of addition of rifampin to PJI surgical treatment, Kruse et al. reported a significant reduction in failure rates when rifampin was used (26.0%) compared with the standard of care (35.9%); the odds ratio was 0.61 (95% confidence interval, 0.43 to 0.86). However, this effect was only seen with exchange arthroplasty and rifampin did not appear useful when implants were retained.

As noted by Kruse et al., for appropriate candidates with PJI, the addition of rifampin to the antibiotic regimen after exchange arthroplasty may improve infection eradication rates.

Ma N, Gogos S, Moaveni A. Do intrawound antibiotics reduce the incidence of surgical site infections in pelvic and lower-limb trauma surgery? A systematic review and meta-analysis. *J Orthop Trauma*. 2022 Nov 1;36(11):e418-24.

What's New in Musculoskeletal Infection

In a systematic review and meta-analysis that focused on patients with skeletal trauma to the lower extremity and the pelvis treated with surgical fixation, Ma et al. examined the effect of the addition of topical vancomycin to intravenous antibiotic therapy. The meta-analysis did not show a significant benefit of topical vancomycin with regard to the reduction of surgical site infections.

Although Ma et al. did not find a significant benefit of using topical vancomycin in their study, further research is necessary to determine whether it may play a role in preventing infection in patients with skeletal trauma treated with surgical fixation.

Xiao M, Money AJ, Pullen WM, Cheung EV, Abrams GD, Freehill MT. Outcomes after resection arthroplasty versus permanent antibiotic spacer for salvage treatment of shoulder periprosthetic joint infections: a systematic review and meta-analysis. *J Shoulder Elbow Surg.* 2022 Mar;31(3):668-79.

Xiao et al. performed a systematic review and meta-analysis comparing patients with shoulder PJI treated with either permanent resection arthroplasty or a permanently retained antibiotic spacer. Although infection eradication rates were similar (82% for the resection arthroplasty and 85% for the antibiotic spacer), patients treated with a permanent antibiotic spacer had significantly better forward flexion and higher American Shoulder and Elbow Surgeons scores.

According to this study, surgeons should make an effort to implant a spacer in patients with chronic shoulder PJI when it is possible to help to maximize function.

What Is the Most Effective Treatment for Periprosthetic Joint Infection After Total Joint Arthroplasty in Patients with Rheumatoid Arthritis?

A Systematic Review

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Abstract

Background: Rheumatoid arthritis (RA) is a risk factor for periprosthetic joint infection (PJI) after total joint arthroplasty (TJA). The purpose of this study was to perform a systematic review comparing the failure rates of debridement, antibiotics, and implant retention (DAIR), one-stage exchange arthroplasty/revision (OSR), and 2-stage exchange arthroplasty/revision (TSR) for RA patients with PJI and identify risk factors in the RA population associated with increased treatment failure rate.

Methods: PubMed, Ovid MEDLINE, and Ovid Embase databases were screened with the terms "rheumatoid arthritis," "total joint arthroplasty," "prosthetic joint infection," and "treatment for PJI" on August 29, 2021. Four hundred ninety-one studies were screened, of which 86 were evaluated. The primary outcome evaluated was failure of surgical treatment for PJI.

Results: Ten retrospective cohort studies were included after full-text screening, yielding 401 patients with RA. Additional demographic and PJI management data were obtained for 149 patients. Patients with RA who underwent TSR demonstrated a lower failure rate (26.8%) than both DAIR (60.1%) and OSR (39.2%) ($\chi^2 = 37.463$, $p < 0.00001$). Patients with RA who underwent DAIR had a 2.27 (95% CI, 1.66-3.10) times higher risk of experiencing treatment failure than those who underwent TSR. Among risk factors, there was a significant difference in the C-reactive protein of patients who did vs. did not experience treatment failure ($p = 0.02$).

Conclusion: TSR has a higher rate of success in the management of PJI patients with RA compared with DAIR and OSR. The complete removal of the infected prosthesis and delayed reimplantation may lower the treatment failure rate.

Level of Evidence: Level III. See Instructions for Authors for a complete description of levels of evidence.

Periprosthetic joint infection (PJI) affects approximately 1% of all patients who undergo total joint arthroplasty (TJA)[1], with significant risk of long-term morbidity and mortality[2,3]. Patients with inflammatory joint diseases such as rheumatoid arthritis (RA) are at a higher risk of developing PJI after TJA[2], reported by 1 study to be as high as 3.7%[2], because of both a higher baseline

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risk of infection and the potential for concurrent immunosuppressive therapies[4,5]. PJI has also been found to develop more rapidly after TJA in patients with RA, with higher rates of polymicrobial PJI[4,6].

Among patients who develop PJI, there are 3 established approaches to treatment: debridement, antibiotics, and implant retention (DAIR), one-stage exchange arthroplasty/revision (OSR), and two-stage exchange arthroplasty/revision (TSR). Current literature indicates that DAIR has a failure rate between 28% to 82%[7-10], but may be more consistently reliable in the setting of acute, rather than chronic, infection[11,12]. Meanwhile, both OSR and TSR have been effective for treating PJI. Although TSR has historically been considered the gold standard[13]—particularly for chronic infection, with a failure rate of 20% or lower, depending on the study[14-17]—OSR has been found to reduce cost[13]. Importantly, several systematic reviews and meta-analyses have not reported statistically significant differences in outcomes when comparing these 2 procedures, potentially suggesting clinical equivalence and need for case-by-case decision-making. However, it is equally important to acknowledge that although the outcomes of these studies were similar, they each included highly selective populations, and thus, results may not be accurately compared across studies[18-22].

Although several reviews have compared the 3 modalities for treatment of post-TJA PJI, no previous study has evaluated these treatments in the RA population. Thus, the purpose of this study was to conduct a systematic review comparing the efficacy of DAIR, OSR, and TSR in treating post-TJA PJI in patients with RA. We additionally sought to identify risk factors that may predispose patients with RA to worse outcomes after PJI. We hypothesized that patients with RA will have lowest failure rates after TSR because their immunosuppressed state may predispose them to more severe infections. We additionally expect immunosuppression status will be associated with increased failure rates.

Methods

Search Strategy, Screening, and Eligibility Criteria

We performed a systematic review comparing PJI outcomes in patients with RA who underwent DAIR, OSR, and TSR after initial total hip arthroplasty (THA) or total knee arthroplasty (TKA), following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and Cochrane collaboration guidelines (Fig. 1; Appendix 1). We searched PubMed, OVID MEDLINE, and OVID Embase databases with specified terms in Appendix 2. This systematic review is exempt from institutional review board approval.

We did not restrict our search by a specified publication date timeframe. Studies were initially excluded if they were duplicates, had titles unrelated to this topic, did not have full-text available, or were presented in a non-English language. After this, studies that included patients with RA who had undergone previous TJA, with subsequent PJI treated operatively by at least 1 surgical procedure of interest, were reviewed in full. Because patients with RA were often a subset of the larger cohort in our included studies, we requested additional data regarding patient characteristics and PJI management from the authors of the eligible studies. Data variables extracted from all studies and from studies responding to our additional data request, respectively, are both found in Appendix 3.

Definitions

DAIR consists of washout, debridement, exchange of modular components to disrupt biofilm, antibiotics, and implant retention[23,24]. TSR consists of a 2-step procedure, as described by Insall et al.[25]. Initially, the prosthesis is removed followed by thorough debridement and irrigation. This is followed either by placement of an antibiotic-laden spacer or beads, or nothing is left behind (Girdlestone procedure). After allowing healing and infection control, the second step consists of prosthesis reimplantation. A similar process may be applied for management of post-THA PJI[26,27]. OSR replicates these steps in a single procedure. Acute PJI was defined as infection within 4 weeks after index arthroplasty[28,29]. It is important to note that several classification systems exist for defining acute PJI; aside from the above definition, other studies have defined acute PJI as infection within 3 months after index arthroplasty[29,30] or divided acute PJI into further subtypes[31,32]. Nonetheless, we elected to use the 4-week time point as it is the most commonly cited value[28,31,33,34]. Chronic PJI was defined as infection after this 4-week period[28,29]. Treatment failure was defined as, within 60 days after PJI treatment, the need for an additional intervention, failure to eradicate infection, infection recurrence, need for chronic antibiotic management, or death because of persistent infection.

Risk-of-Bias Assessment

Two independent reviewers assessed the risk of bias within each included randomized trial. Bias was analyzed through the Cochrane Risk-of-Bias Assessment Tool: for Non-Randomized Studies of Interventions for cohort and case–control studies (Appendix 4). Discordance between reviewers was settled by a third reviewer.

Statistical Analysis

Rate of failure after surgical treatment was determined by dividing the number of patients who failed treatment per technique by the total number of patients who underwent each approach. Continuous variables for patient demographics were reported as median and range, whereas categorical data were presented as frequency variables with percentages per race group. Kruskal-Wallis nonparametric testing was used to evaluate statistical significance for continuous variables, and χ^2 tests were used for categorical variables. Difference in failure rate was assessed using a χ^2 test. A generalized linear regression model was created to quantify the association of patient characteristic variables and likelihood of failure. Patients with missing

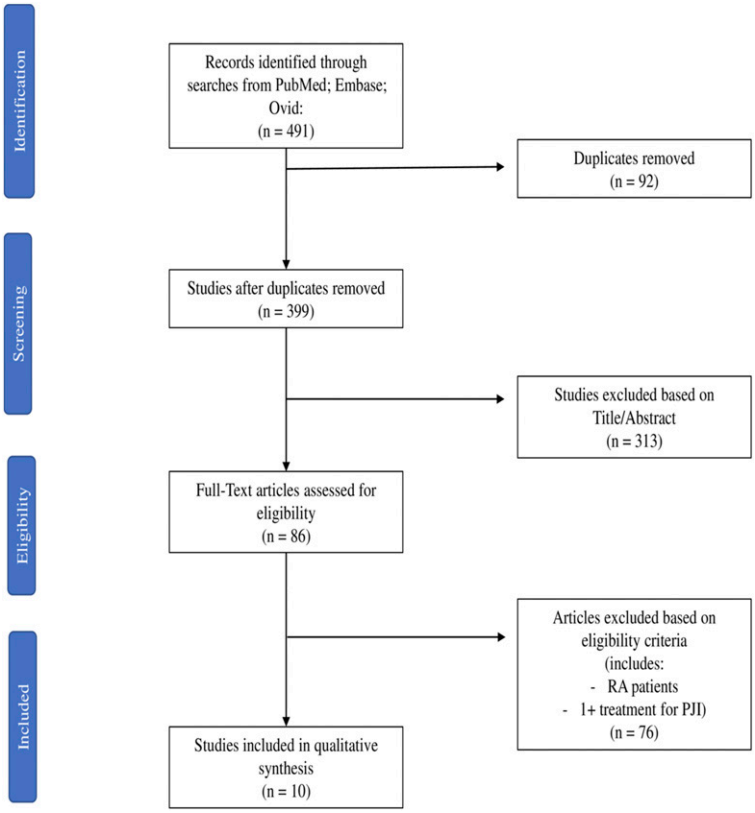

Fig. 1

PRISMA flow diagram of the literature search process. PJI = periprosthetic joint infection, PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses, and RA = rheumatoid arthritis.

values were excluded from analysis. Analyses were performed using R Statistical Software (version 4.1.2; 2021; R Core Team) and JMP Pro version 17.0.0 (SAS Institute). An alpha value of 0.05 was chosen for significance.

Results

Study Selection and Characteristics

The initial search revealed 491 studies, with 92 duplicates and 313 studies removed based on initial screening. Eighty-six studies remained for full-text review, after which 10 studies remained eligible (Fig. 1; Table I). These studies included 9 retrospective cohort studies[4,35-42] and 1 prospective cohort study[43]. Four hundred one patients with RA across these 10 studies were included. Four studies provided additional demographic and PJI management data on request[36,40-42].

Demographics and Patient Characteristics

Additional patient demographic and PJI management data requests yielded a total of 149 patients (37.2% of overall cohort, Table III). Among this cohort, 71.1% of patients were female, median age was 69.0 (range, 45-93) years, and median Charlson Comorbidity Index (CCI) was 3.0 (range, 0.0-9.0). Approximately 28.2% of patients initially underwent THA, 31.5% of patients underwent TKA, and 40.3% did not report the involved joint. The most common surgical technique performed for PJI in this cohort was DAIR (85.2%), followed by TSR (10.1%), OSR (2.0%), and not reported (3.5%). Among patients who failed reoperation, median time to reoperation after initial PJI was 225.0 days.

Outcome of Surgical Treatment

Among the overall 401 patients with RA who sustained PJI after TJA, 204 patients (50.87%) underwent DAIR, 74 patients (18.45%) underwent OSR, and 123 patients (30.67%) underwent TSR (Table II). Those who sustained PJI and underwent TSR demonstrated a lower failure rate (26.8%) than both DAIR (60.1%) and OSR (39.2%). The relationship between treatment strategy and outcome was statistically significant ($\chi^2 = 37.46$, $p < 0.00001$), meaning that the failure rate was significantly different among the 3 treatment strategies.

Within our overall cohort of 401 patients, DAIR demonstrated a higher failure rate than TSR ($\chi^2 = 35.44$, $p < 0.0001$). Patients with RA who underwent DAIR had a 2.27 (RR, 2.27; 95% CI, 1.66-3.10; $p < 0.001$) times higher risk of experiencing treatment failure than those who underwent TSR. Similarly, patients who underwent DAIR had a higher failure rate than patients who received OSR ($\chi^2 = 10.23$, $p = 0.0014$). Patients in the DAIR cohort had a 1.55 (95% CI, 1.14-2.10; $p < 0.001$) times higher risk of experiencing treatment failure than patients in the OSR cohort. There was no statistically significant difference in failure rate between the TSR and OSR cohorts ($\chi^2 = 3.273$, $p = 0.07$).

In our smaller cohort of 149 patients, we stratified patients by acute vs. chronic PJI. One hundred twelve of

TABLE I Summary of Included Studies*

Study	Year	Design	Treatments Included	Duration of Follow-up	Results	Limitations
Lora-Tamayo et al.[42]	2013	RC	DAIR	24 months	• Patients with RA had a significantly greater odds of experiencing early failure (failure within 30 days of debridement) after DAIR (adjusted OR, 3.88 [1.44-10.4], p = 0.007) • Patients with RA did not have a significantly greater odds of experiencing late failure (failure after 30 days after debridement while on antibiotic therapy) or failure after therapy (failure after end of antibiotic therapy) • Patients with RA had a 66% rate of failure after DAIR • Patients with RA had a significantly higher risk of experiencing overall failure (HR, 1.84 [1.14-2.99], p = 0.021)	• Retrospective study
Lora-Tamayo et al.[41]	2017	RC	DAIR	802 days (median)	• Patients with RA had a significantly higher risk of experiencing overall failure (HR, 2.36 [1.50-3.72], p < 0.01) • Patients with RA had a 65% rate of failure after DAIR • Patients with RA had a significantly greater odds of experiencing early failure (failure within 30 days of debridement) after DAIR (adjusted OR, 3.33 [1.40-7.93], p = 0.007) • Patients with RA did not have a significantly greater odds of experiencing late failure (failure after 30 days after debridement while on antibiotic therapy) or failure after therapy (failure after end of antibiotic therapy)	• Retrospective study • Patient management with DAIR across the included institutions was not standardized

Hsieh et al.[4]	2013	RC	DAIR; TSR	24 months	• Percent of patients with RA who underwent each procedure:
					  • 46% DAIR (21/46 patients)
					  • 61% TSR (28/46 patients)
					• Percent of RA patients with PJI that experienced treatment failure with each procedure:
					  • 76% DAIR (16 patients)
					  • 25% TSR (7 patients)
						• Retrospective study

Berbari et al.[38]	2006	RC	DAIR; TSR; OSR	5 years	• Percent of RA patients with PJI that experienced treatment failure with each procedure:
					  • 67% DAIR (15/46 patients)
					  • 21% TSR (8/39 patients)
					  • 39% OSR (29/74 patients)
					• Patients with RA who received DAIR had a greater risk of experiencing failure in comparison with those who received TSR (HR, 5.7 [2.6-13.4], p < 0.001)
					• Patients with RA who received OSR had a greater risk of experiencing failure in comparison with those who received TSR (HR, 2.5 [1.2-2.7], p = 0.03)
						• Retrospective study
						• Procedure treatment protocols were not standardized

Kuiper et al.[40]	2013	RC	DAIR	35 months (mean)	• Patients with RA had a 70% rate of failure after DAIR • Patients with RA had a significantly greater odds of experiencing failure after DAIR (OR, 1.2-84, p = 0.03)	• Retrospective study • Patient management with DAIR across the included institutions was not standardized • Small sample size

continued

TABLE I (*continued*)

Study	Year	Design	Treatments Included	Duration of Follow-up	Results	Limitations
Hirakawa et al.[37]	1998	RC	TSR	61.9 months (mean)	• Patients with RA had a 46% rate of failure after TSR	• Retrospective study
Rajgopal et al.[35]	2018	RC	TSR	5.3 years (mean)	• Patients with RA had a 44% rate of failure after TSR • The odds of failure were significantly higher in patients with RA (OR, 3.94 [1.42-11.88], p = 0.008)	• Retrospective study
Löwik et al.[36]	2018	RC	DAIR	60 days	• Patients with RA had a 39% rate of failure after DAIR • RA was not associated with a significant difference in failure rate after DAIR (p = 0.915)	• Retrospective study
Grzelecki et al.[43]	2018	PC	TSR	53.3 months (mean)	• Patients with RA had a 20% rate of failure after TSR • RA was not associated with a significant difference in failure rate after TSR (p = 0.60)	

Singh et al.[39]	2019	RC	DAIR; TSR	2 years	• Percent of patients with RA who underwent each procedure:
					  • 79% DAIR (33/42 patients)
					  • 21% TSR (9/42 patients)
					• Percent of RA patients with PJI that experienced treatment failure with each procedure:
					  • 48% DAIR (16/33 patients)
					  • 11% TSR (1/9 patients)
					• Patients with RA who underwent DAIR had a significantly greater risk of experiencing treatment failure in comparison with those who underwent TSR (HR, 4.42 [2.58-7.57])
						• Retrospective study

*DAIR = debridement, antibiotics, and implant retention, HR = hazard ratio, OR = odds ratio, OSR = one-stage exchange arthroplasty, PC = prospective cohort, PJI = periprosthetic joint infection, RA = rheumatoid arthritis, RC = retrospective cohort, and TSR = two-stage exchange arthroplasty.

the 149 patients had data recorded for PJI chronicity, procedure type, and outcome. Among the 88 patients who sustained acute PJI, the failure rate among the 3 treatment strategies was significantly different ($\chi^2 = 6.23$, p = 0.04). Furthermore, within the acute PJI cohort, the treatment failure rate of DAIR (64.9%) was statistically significantly higher than that of TSR (22.2%) ($\chi^2 = 6.15$, p = 0.01). We were unable to perform analysis on the 24 chronic PJI patients because of small sample size.

PJI Characteristics

One hundred thirty-one patients (87.9%) of the 149-patient cohort had data available regarding infectious pathogen. The most common pathogen identified in the cohort was *Staphylococcus* spp. (54.2%), followed by *Streptococcus* spp. (26.0%), and polymicrobial infections (9.9%). Methicillin-sensitive *S. aureus* (MSSA, 19.8%), methicillin-resistant *S. aureus* (MRSA, 6.1%), and coagulase-negative *Staphylococcus* (10.7%) were the common *Staphylococcus* organisms, whereas 22.1% of the cohort had an unspecified *S. aureus* infection.

Likelihood and Risk Factors for PJI Treatment Failure

Approximately 116 patients of the 149-patient cohort had recorded data regarding both PJI chronicity and the surgical treatment performed (Table III). Among the patients who underwent DAIR, 78 (78.0%) had sustained an acute PJI, and 22 (22.0%) had sustained a chronic PJI. Nine (69.2%) patients who underwent TSR had sustained acute PJI, whereas 4 (30.8%) patients had sustained chronic PJI. Finally, the 2 patients in this cohort who underwent OSR had sustained acute PJI.

In this cohort of 116 patients, we evaluated the effect of age, sex, CCI, immunosuppressant therapy, surgical technique (including DAIR, OSR, and TSR), type of PJI (acute vs. chronic), and site of implant on likelihood of PJI treatment failure. We report no statistically significant impact of any of these characteristics on likelihood of PJI treatment failure (Table IV). When evaluating only patients who underwent DAIR in this cohort, there was similarly no evidence, suggesting a significant difference in failure rate between patients with acute vs. chronic PJI ($\chi^2 = 0.62$, p = 0.89).

TABLE II Data for Total Number of Treatments Performed and Number of Treatment Failures Across the 10 Included Studies*

Study No.	Study	Total No. of Procedures on Patients with RA	Treatments Evaluated	No. Failed (DAIR)	Total (DAIR)	No. Failed (TSR)	Total (TSR)	No. Failed (OSR)	Total (OSR)
1	Rajgopal et al. (2018)[35]	18	DAIR + TSR; TSR direct	0	0	8	18	0	0
2	Löwik et al. (2018)[36]	28	DAIR	11	28	0	0	0	0
3	Grzelecki et al. (2019)[43]	15	TSR	0	0	3	15	0	0
4	Singh et al. (2019)[39]	42	DAIR and TSR	16	33	1	9	0	0
5	Lora-Tomayo et al. (2013)[42]	29	DAIR	19	29	0	0	0	0
6	Lora-Tomayo et al. (2017)[41]	37	DAIR	24	37	0	0	0	0
7	Hsieh et al. (2013)[4]	49	DAIR and TSR	16	21	7	28	0	0
8	Berbari et al. (2006)[38]	159	DAIR, TSR, and OSR	31	46	8	39	29	74
9	Hirakawa et al. (1998)[37]	14	TSR	0	0	6	14	0	0
10	Kuiper et al. (2013)[40]	10	DAIR	7	10	0	0	0	0
	Total	401		124	204	33	123	29	74
	Failure rate (%)				60.8		26.8		39.2

*DAIR = debridement, antibiotics, and implant retention, OSR = one-stage exchange arthroplasty, RA = rheumatoid arthritis, and TSR = two-stage exchange arthroplasty.

There was a significant difference in the C-reactive protein (CRP) at initial presentation after index arthroplasty among patients who experienced treatment failure (median 158.5 mg/L; interquartile range [IQR], 59.8-306.8, range, 2-596) in comparison with patients who did not experience treatment failure (median 109 mg/L; IQR, 38.5-222.5, range, 0-390) ($p = 0.02$). The median white blood cell (WBC) count was 10,300/mL (IQR, 7,300-14,550, range, 3,600-27,100) for patients who experienced treatment failure and 10,500/mL (IQR, 8,300-14,500, range, 9-26,500) for those who did not experience treatment failure ($p = 0.28$).

Failure in Total Hip vs. Total Knee Arthroplasty Patients

Approximately 42 patients (28.8% of initial cohort) underwent initial THA, whereas 47 patients (31.1%) underwent TKA. There was no statistically significant difference in sex (69.0% vs. 74.5% female; $p = 0.74$), age (median 75.5 [IQR, 63.50-82.00] vs. 69.0 [63.50-75.00]; $p = 0.125$), race ($p = 0.645$), or pathogen type ($p = 1.00$) between these cohorts. Median joint age by time of presentation with PJI was 22.5 days (IQR, 16.00-75.00) among patients who underwent initial THA vs. 343.0 days (IQR, 20.00-1,562.00) among patients who underwent initial TKA ($p = 0.013$). Type of PJI significantly differed between the 2 groups ($p < 0.001$), with 12 post-THA patients (50.0% of available data) vs. 26 post-TKA patients (81.3% of available data) presenting with acute PJI ($p < 0.001$). We additionally report a statistically significant difference in presenting CRP (median 81.00 mg/L [IQR, 37.00-272.00] vs. 208.00 mg/L [IQR, 107.50-304.50]; $p = 0.049$) between post-THA and post-TKA cohorts, respectively. There is no statistically significant difference between presenting ESR (median 82.00 mm/H [IQR, 70.50-219.50] vs. 95.00 mm/H [IQR, 77.00-99.50]; $p = 0.732$), presenting WBC (median 11,300.00/mL [IQR, 7,950.00-15,900.00] vs. 10,550.00/mL [IQR, 8,125.00-14,250.00]), or immunosuppression status (16 patients [44.4%] vs. 25 patients [55.6%] on immunosuppression; $p = 0.441$).

Among the 42 patients who underwent initial THA and 47 patients who underwent initial TKA, 21 (50.0%) and 24 (51.1%), respectively, failed post-PJI management ($p = 1.00$). Among this subset of patients, there was no statistically significant difference in sex (61.9% [THA] vs. 83.3% [TKA] female; $p = 0.199$), age (median 76.0 [IQR, 63.0-82.0] years vs. 73.0 [IQR, 62.75-79.50] years; $p = 0.758$), race ($p = 0.327$), joint age ($p = 0.619$), or pathogen type ($p = 1$) between THA and TKA cohorts. Furthermore, we report no statistically significant difference in clinical characteristics between these 2 cohorts, including presenting CRP (median 132.00 mg/L [IQR, 75.25-312.00] vs. 258.50 mg/L [IQR, 131.50-315.00]; $p = 0.291$), presenting ESR (median 82.00 mm/H [IQR, 81.00-200.00] vs. 104.00 mm/H [IQR, 104.00-104.00]; $p = 0.77$), presenting WBC (median 11,450.00/mL [IQR, 7,275.00-15,875.00] vs. 10,600.00/mL [IQR, 8,200.00-13,700.00]; $p = 0.886$), type of PJI ($p = 0.108$), and immunosuppression status (7 [41.2%] vs. 12 [52.2%] on immunosuppression; $p = 0.713$). The most commonly used surgical technique was DAIR in both cohorts (16 patients, 76.2% of the failure-post-THA cohort; 19 patients, 79.2% of the failure-post-TKA cohort) with no statistically significant difference in overall use of surgical technique between the 2 cohorts ($p = 0.24$).

TABLE III Additional Demographic and PJI Management Data Acquired from 4 Studies' Authors for 149 Patients of the Overall RA Patient Cohort*

Variable	Outcome of Surgical Treatment		p Value
	Failure (n = 86)	No Failure (n = 63)	
Age	69.3 (45-93)	69.0 (48-89)	0.90
Sex			
Female	70.9% (n = 61)	71.4% (n = 45)	0.95
Male	29.1% (n = 25)	28.6% (n = 18)	
Joint			
Hip	24.4% (n = 21)	33.3% (n = 21)	0.92
Knee	27.9% (n = 24)	36.5% (n = 23)	
Not reported	47.7% (n = 41)	30.2% (n = 19)	
CRP (mg/L)	192 (2-596)	97.5 (0-390)	0.02†
Procedure			
DAIR	88.4% (n = 76)	81.0% (n = 51)	0.14
1-stage	2.3% (n = 2)	1.6% (n = 1)	
2-stage	5.8% (n = 5)	15.9% (n = 10)	
Not reported	3.5% (n = 3)	1.6% (n = 1)	
Type of PJI			
Acute	61.6% (n = 53)	55.6% (n = 35)	0.90
Chronic	19.8% (n = 17)	17.5% (n = 11)	
Not reported	18.6% (n = 16)	27.0% (n = 17)	
Pathogen			
S. aureus	19.8% (n = 17)	19.0% (n = 12)	0.09
MSSA	15.1% (n = 13)	20.6% (n = 13)	
MRSA	7.0% (n = 6)	3.2% (n = 2)	
S. aureus, polymicrobial	4.7% (n = 4)	6.3% (n = 4)	
Streptococcus spp.	23.3% (n = 20)	14.3% (n = 9)	
Streptococcus spp., polymicrobial	5.8% (n = 5)	0.0% (n = 0)	
CoNS	4.7% (n = 4)	15.9% (n = 10)	
Other	9.3% (n = 8)	6.3% (n = 4)	
Not reported	10.5% (n = 9)	14.3% (n = 9)	
Median joint age	225 (0-8,941)	48 (3-8,128)	0.33
CCI	3.0 (0-9)	3.0 (0-9)	0.96
WBC	10,300 (3,600-27,100)	10,500 (9,000-26,500)	0.28

*CCI = Charlson Comorbidity Index, CoNS = coagulase-negative *Staphylococcus*, CRP = C-reactive protein, DAIR = debridement, antibiotics, and implant retention, MSSA = methicillin-sensitive *Staphylococcus aureus*, MRSA = methicillin-resistant *Staphylococcus aureus*, PJI = periprosthetic joint infection, and WBC = white blood cell.
†Statistically significant.

Risk of Bias

Overall, the 9 retrospective cohort studies and 1 prospective cohort study had a low risk of bias (Table V).

Discussion

Patients with RA pose a challenge for surgeons because of their chronic inflammatory state and the increased risk of treatment failure after PJI[35,40]. This study compared failure rates of 3 major surgical interventions for PJI in patients with RA who underwent TJA and evaluated factors that may affect both failure rate and likelihood of failure. In our larger cohort, our results showed that both TSR and OSR had a higher rate of success for acute PJI management than did DAIR. In our smaller cohort, in which PJI chronicity data were available, TSR was found to be significantly more effective than DAIR in the acute PJI population. Outcomes were similar between post-THA and post-TKA patients. Additional analyses revealed, interestingly, immunosuppressive therapy status did not significantly affect likelihood of treatment failure.

Although DAIR was most commonly performed, our results suggest that TSR is a more effective first-line treatment in this patient population. This is consistent with the widely reported finding that TSR is the gold standard of chronic PJI treatment[44-46].

TABLE IV General Linear Model to Identify Effect of Variables on Outcomes After Surgical Management of PJI, Presented as an OR*

Characteristic	Likelihood of Treatment Failure After Surgical Intervention (OR; 95% CI)	p Value
Age	0.898 (0.747 to 1.049)	0.1630
Male sex	0.831 (−0.800 to 2.462)	0.8243
CCI	3.091 (1.860 to 4.321)	0.0722
Immunosuppressant therapy	0.828 (−0.879 to 2.535)	0.8282
Surgical technique: DAIR	0 (−10 to 10)	0.9953
Surgical technique: OSR	0 (−10 to 10)	0.9949
Surgical technique: TSR	0 (−10 to 10)	0.9948
Type of PJI: acute	0.482 (−2.016 to 2.979)	0.5667
Type of PJI: chronic	1.276 (−1.928 to 4.481)	0.8813
Site: knee	1.283 (−0.728 to 3.295)	0.8081

*CCI = Charlson Comorbidity Index, DAIR = debridement, antibiotics, and implant retention, OR = odds ratio, OSR = one-stage revision, and TSR = two-stage revision.
†Statistically significant.

Importantly, these studies have not been performed specifically in the RA patient population. However, given that patients with RA have a higher baseline risk of PJI[4,40], it is likely that these reports remain applicable; studies have shown that TSR is particularly effective in patients with resistant organisms, suggesting higher efficacy against more severe infection to which patients with RA may be more prone[4,47-52]. This assertion is ultimately supported by our findings, stating that, although success rates for all procedures were lower in the RA patient population than in the general population, TSR is substantially more effective in this patient cohort over DAIR—perhaps because of the removal of the prosthesis that allows for more thorough debridement, reducing biofilm and microbial burden[4,38]. As stated above, it is important to note that, historically, TSR has been used for more severe infections[51,52]; infection severity is determined at the individual patient level and is not able to be addressed in our review, given that it comprisesf more retrospective studies. If TSR was more commonly used for patients presenting with more severe infections—meaning these patients are more likely to fail treatment—it is possible that there is an even more significant difference in likelihood of failure between TSR and DAIR than was reported in this study. A valuable avenue for further research is further evaluation of OSR in this population, considering the established benefits of a single procedure, a shorter antibiotic course, and decreased cost[13]; results from the larger cohort demonstrated OSR may be equivalent to TSR in patients with RA; however, limited number of patients undergoing OSR in our smaller data set precluded further analysis.

Interestingly, our results suggest that TSR has potential efficacy even in

TABLE V Consensus ACROBAT-NRSI Judgments Between 2 Reviewers by Domain of Bias of Included Cohort Studies

Study	D1: Bias Because of Confounding	D2: Selection of Participants	D3: Bias in Measurement of Interventions	D4: Bias Because of Departure from Intended Intervention	D6: Bias Because of Missing Data	D7: Bias in Selection of Reported Results	Overall Risk-of-Bias Assessment
Lora-Tamayo et al. (2013)[42]	Low	Low	Low	Low	Low	Low	Low
Lora-Tamayo et al. (2017)[41]	Low	Moderate	Low	Low	Low	Low	Low
Hsieh et al.[4]	Low	Low	Low	Low	Low	Low	Low
Berbari et al.[38]	Low	Low	Low	Low	Low	Low	Low
Kuiper et al.[40]	Low	Low	Low	Low	Low	Low	Low
Hirakawa et al.[37]	Low	Low	Low	Low	Low	Low	Low
Rajgopal et al.[35]	Low	Low	Low	Low	Low	Low	Low
Löwik et al.[36]	Low	Low	Low	Low	Low	Low	Low
Grzelecki et al.[43]	Low	Low	Low	Low	Low	Low	Low
Singh et al.[39]	Low	Low	Low	Low	Low	Low	Low

the acute setting; however, current literature on non-RA patient populations indicates that DAIR should be used the first-line option for infection in this context, given decreased morbidity, difficulty of surgery, and biofilm burden at that time[34,53-57]. Ultimately, further research is needed to perform a more robust comparison of DAIR vs. TSR in the acute setting in the RA population, considering their higher propensity for severe infection, before providing definitive recommendations.

Patients with RA are reported to have worse outcomes after post-TJA PJI in comparison with those in patients without RA[58-60], suggested by several studies to be due to baseline inflammatory processes and immunosuppression that predisposes patients to earlier infection and ultimate joint failure[6,61,62]; however, this remains debated[62]. In this study, we reported that immunosuppression was not significantly associated with likelihood of PJI treatment failure in patients with RA. Nonetheless, immunosuppressive therapy likely still plays a role in predisposing patients with RA to PJI treatment failure, in addition to chronic underlying inflammatory processes related to RA. In support of the latter, we found a significant difference in CRP on admission between patients who did and did not experience treatment failure. However, elevated CRP among those who failed treatment may be representative of a more aggressive infectious process rather than of the inflammatory process chronically underlying RA. Other current theories regarding increased susceptibility of patients with RA to sustaining PJI allude to the role of the underlying autoimmune disease itself or the persistence of bacteria in the joint space that were not previously detected[6].

There are several limitations to this study. First, we were restricted by the available studies on a relatively narrow topic. Several studies included patients with RA as a subset of their analyses, and thus, many did not provide information about patient characteristics that were required for our subanalyses. We ultimately obtained sufficient data for 149 patients of our overall 401-patient cohort for further analysis. Second, it is pertinent to note that not all the additional data we received from the included studies reported on the same patient characteristics. The low sample sizes for several factors we assessed contributed potentially to nonsignificant associations between these factors—for example, small number of patients undergoing OSR—and treatment failure. Relatedly, because these additional data were aggregated from different clinical sites, these data are susceptible to selection, indication, and surveillance bias. Most importantly, we are unable ascertain indications used for treatment decision-making, which may significantly impact outcomes and present a source of confounding to our study; an example previously provided was if patients undergoing TSR had more severe infection on presentation that those undergoing DAIR or OSR, perhaps, our results are understating the superiority of TSR. Because we are unable to determine whether the indications for use of DAIR were standardized across included studies, it must be assumed that those who underwent DAIR were chosen appropriately in each cohort and thus adequately represent the merits of this procedure. Similarly, as we were unable to determine whether DAIR treatment protocols were standardized across each included study—particularly regarding exchange of modular components, which has been reported as an independent predictor of treatment success in patients without RA[41]—we cannot provide definitive recommendations on the efficacy of DAIR in the RA population. Third, 3.5% of patients did not have procedure type listed; given that OSR was only 2% of our cohort, data from this 3.5% may have affected our results. To mitigate this effect, once finding that these data could not be imputed, we discarded this subset of incomplete data from our analyses. Fourth, we were unable to stratify based on type of immunosuppressant medication; it is possible that use of immunomodulators vs. biologics, for example, may have distinct outcomes. Fifth, we elected to define treatment failure at 60 days because this was the most commonly used value among included studies, maximizing inclusion of the already-limited available data; however, this is a potential source of bias, given that infection may persist beyond this point. Similarly, as described in Methods, we used a 4-week cutoff for acute infection as it was most commonly cited[28,31,33,34]; however, use of this definition, which includes a relatively wide timeframe, may also introduce a potential source of bias. Last, we only assessed a limited spectrum of comorbidities, and social factors such as alcohol consumption and smoking were not captured in the CCI.

Overall, our study determined that TSR had the highest rate of success among the 3 most commonly performed procedures for PJI management in patients with RA. When stratifying patients by chronicity in our smaller cohort, TSR continued to demonstrate a lower rate of failure than did DAIR. Thus, we propose consideration of TSR, or perhaps at least reconsideration of electing DAIR, for all RA patients with PJI, including after both THA and TKA, particularly in the chronic setting. The use of DAIR, given its advantages of lower morbidity and decreased technical demand compared with the alternative procedures[56,57], should still be used in the correct clinical context, particularly among patients for whom extensive surgery may be risky or in the acute setting when biofilm has yet to be formed. It is important to emphasize that these findings and conclusions should be considered in context of the limitations listed above. It is our hope that these results may provide a better understanding of treatment options to help surgeons and patients with RA engage in shared decision-making to optimize management of PJI. Future studies may benefit from comparing RA PJI patients with non-RA PJI patients to determine whether differences in surgical management are true between varying patient populations.

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No funding was obtained for this study.

Appendix

Supporting material provided by the authors is posted with the online version of this article as a data supplement at jbjs.org (http://links.lww.com/JBJSREV/B60). This content was not copyedited or verified by *JBJS*.

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Commentary & Perspective

Will Preoperative Synovial Fluid Antigen Testing Change Our Clinical Practice?

Commentary on an article by Krista O'Shaughnessey Toler, MS, MBA, PMP, et al.: "Nationwide Results of Microorganism Antigen Testing as a Component of Preoperative Synovial Fluid Analysis"

Marjan Wouthuyzen-Bakker, MD, PhD

In their article, Toler et al. evaluated the diagnostic performance of a recently launched synovial fluid antigen test for the preoperative diagnosis of periprosthetic joint infection (PJI). The test is of great interest because identifying the causative microorganism prior to the surgical procedure is important in guiding surgical decision-making and starting targeted antimicrobial treatment as soon as possible. Toler et al. demonstrated a high concordance between synovial fluid culture and the antigen test. Moreover, in culture-negative synovial fluids, the test identified a microorganism in 49% of cases. However, from a clinical point of view, there are important limitations that should be taken into consideration.

With regard to how the test will guide antibiotic treatment, the following shortcomings should be taken into account. A limited number of species (i.e., Staphylococcus, Enterococcus, and Candida species) are included in the test, as noted in the article, and it is important to keep in mind that the reference test used to calculate the diagnostic accuracy was a positive synovial fluid culture for the included species. A comparison with intraoperative tissue cultures was not performed, and, because the sensitivity of synovial culture is poor, an infection cannot be ruled out in the case of a negative antigen test. Therefore, empirical antibiotic treatment should still be administered in the case of a negative antigen test when a PJI is suspected. In the case of a positive test, it is important to realize that, in addition to the inclusion of a limited number of species that can be detected, the test only identifies species on a genus level. Because the antibiotic treatments for methicillin-sensitive staphylococci compared with methicillin-resistant staphylococci and for *Enterococcus faecalis* compared with *Enterococcus faecium* are different, their identification on a genus level cannot fully target antibiotic treatment. Other tests that overcome this limitation are available, such as the recently launched multiplex polymerase chain reaction (PCR) for bone and joint infections[1]. The BioFire Joint Infection PCR Panel provides a rapid diagnosis (i.e., within 1 hour), includes more genera and identifies pathogens on a species level, and detects resistance genes, which may better guide antimicrobial treatment[1]. Also, other microorganisms not detected by the PCR test may be involved. According to the literature, around 30% to 40% of PJIs are polymicrobial in nature, with an even higher incidence observed in early postoperative and chronic infections with a sinus tract[2,3]. Consequently, empirical antibiotic treatment cannot be easily narrowed in the case of a positive antigen test either.

With regard to how the test will help us in surgical decision-making, some experts in the field have advocated a 2-stage exchange instead of 1-stage exchange for PJI caused by difficult-to-treat microorganisms such as enterococci and Candida[4,5]. Therefore, isolating these species prior to the surgical procedure may guide surgical decision-making. However, in the total cohort in the study by Toler et al., Candida species already grew on synovial fluid culture in 338 cases and Enterococcus species grew on culture in 465 cases. More rapid identification that the antigen provides is not needed in chronic infections, as one has time to wait for the final culture results. In the remaining preoperative samples with negative synovial fluid cultures, the antigen test detected Candida in an additional 142 cases and enterococci in an additional 188 cases. If these extra cases were to be considered to represent real infections, rather than false-positives as stated by Toler et al., only 0.3% of candidal infections and 0.4% of enterococcal infections from the total cohort of patients will have been missed when solely relying on preoperative cultures of synovial fluid. This low percentage of missed infections makes one wonder whether the number needed to test is really justified.

Last but not least, Toler et al. analyze their results according to whether the preoperative diagnosis, according to the 2018 International Consensus Meeting, was positive or negative, but the inconclusive cases (7.5% of the total cohort) are not included in the final analysis, although these are the cases that are most clinically challenging.

Based on the above-mentioned limitations and the way that the results are analyzed, it seems that the evaluated antigen test will not change our daily clinical practice in a large number of cases. Additional analyses, particularly comparisons with the final postoperative diagnosis and intraoperative culture results, are needed to define the role of this test in the diagnostic workup.

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Current Concepts Review

The Challenge of Emerging Resistant Gram-Positive Pathogens in Hip and Knee Periprosthetic Joint Infections

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- An increase in resistant bacterial pathogens has occurred over the last 4 decades.
- Careful patient selection and improving or correcting risk factors for periprosthetic joint infection (PJI) before elective surgical treatment are strongly recommended.
- Appropriate microbiological methods, including those used to detect and grow *Cutibacterium acnes,* are recommended.
- Antimicrobial agents used in the prevention or management of infection should be selected appropriately and the duration of therapy should be carefully considered in order to mitigate the risk of developing bacterial resistance.
- Molecular methods including rapid polymerase chain reaction (PCR) diagnostics, 16S sequencing, and/or shotgun and/or targeted whole-genome sequencing are recommended in culture-negative cases of PJI.
- Expert consultation with an infectious diseases specialist (if available) is recommended to assist with the appropriate antimicrobial management and monitoring of patients with PJI.

Historical Perspective

Total hip arthroplasty (THA) and total knee arthroplasty (TKA) are among the most successful surgical procedures in medicine[1,2]. Despite the tremendous success of hip and knee joint replacement, complications such as periprosthetic joint infection (PJI) can adversely affect the outcome[1]. Four decades ago, Sir John Charnley[2] stated: "Postoperative infection is the saddest of all complications." The challenge of managing PJI is made more difficult if the bacteria associated with the infection are resistant to antibiotics. Antimicrobial resistance is not a new problem, having been recognized soon after the discovery of penicillin. Penicillin was first identified in 1929 and, by 1941, was used commonly as the antibiotic to successfully treat *Staphylococcus aureus*[3]. Widespread resistance to penicillin necessitated new antibiotic development, leading to the discovery of methicillin in the late 1950s. Unfortunately, within a few years, the first case of methicillin-resistant *S. aureus* (MRSA) was reported[4]. MRSA in PJI was not reported until much later[5,6]. MRSA, Enterococcus, and gram-negative bacteria were all identified as virulent pathogens associated with a higher risk of failure after 2-stage reimplantation for PJI[7,8].

There has been an increase in MRSA-related PJI in the field of arthroplasty[9,10]. Parvizi et al. reported that 34% of PJIs between 2002 and 2007 were due to MRSA or methicillin-resistant *Staphylococcus epidermidis* (MRSE)[9]. Aggarwal et al. compared pathogens in PJI between 2 large infection referral centers in the United States and Germany and reported that 48.1% of *S. aureus* infections in the United States were MRSA compared with 12.8% in Europe, and the rate of vancomycin-resistant Enterococcus (VRE) was 0% in Europe and 26.7% in the United States[10]. In a retrospective study of 937 PJIs from 2003 to 2011 in Germany, Rosteius et al. discovered an

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increased incidence in the number of PJIs due to multidrug-resistant organisms[11]. In this cohort, MRSA was the second most common infectious organism and MRSE was the third most common. The prevalence of resistance in PJIs has persisted[12,13].

The purposes of this study were to provide data on the prevalence of antibiotic resistance in gram-positive bacterial pathogens, discuss methods for surgeons to better identify and manage their patients who have developed antimicrobial-resistant PJI, and, finally, provide information intended to help us to develop the means to slow the emergence of antimicrobial-resistant PJIs.

Mechanism for Pathogen Resistance

The inability to adequately treat infections is primarily due to a variety of host and bacterial factors[14]. Particularly relevant to foreign body infections is the ability of bacteria to produce a biofilm on the surface of the device[15]. It is pertinent to note that biofilms yield unique challenges related to antibiotic resistance, as many biofilm-associated bacteria are quiescent and thus do not respond to many antibiotics as they would if growing in a planktonic state[16-18]. Therefore, this typically necessitates foreign body removal, as bacteria growing in a biofilm are not necessarily sterilized using systemic antibiotics. Furthermore, recent evidence has found that the major leukocyte infiltrate during PJIs is granulocytic myeloid-derived suppressor cells (which suppress T-cell recruitment and proinflammatory cytokine production at the site of infection, further repressing an acute inflammatory response[19]). The inability to sterilize colonized foreign bodies with antibiotics generally requires removal of the implant, with an increased potential for morbidity. Further complicating antibiotic treatment is the concept of bacterial persistence, which is linked to multiple mechanisms[20]. However, this section will focus on specific acquired mechanisms of resistance to antibiotics commonly used to treat orthopaedic infections caused by gram-positive pathogens[21,22].

S. aureus *and Other Staphylococcus Species*

The discovery of penicillin had a substantial impact on the treatment of serious gram-positive infections, including those caused by *S. aureus*. However, approximately 90% to 95% of all current clinical *S. aureus* isolates are resistant to penicillin due to the acquisition of plasmids encoding a penicillinase[3,23,24]. Semisynthetic penicillins (e.g., methicillin) that are not cleaved by the staphylococcal penicillinase were developed in the late 1950s[25]. Soon after the introduction and use of methicillin as an antibacterial agent, *S. aureus* and other coagulase-negative staphylococci were isolated that were resistant. Later studies found that resistance was due to the acquisition of a new penicillin-binding protein (PBP), called PBP2A[26]. In conjunction with native PBPs, organisms that have acquired PBP2A are able to synthesize a cell wall in the presence of semisynthetic penicillins, resulting in a resistant phenotype.

Vancomycin remains the standard therapy for the treatment of MRSA infections, and non-susceptibility to vancomycin has remained rare. Intermediate resistance to vancomycin (vancomycin intermediate *S. aureus* [VISA]) is typically observed in patients undergoing long-term vancomycin therapy[27]. The role of newer lipoglycopeptides[28-31] in the treatment of patients infected with VISA is isolate-dependent and, therefore, susceptibility testing should be performed to determine if the isolate is susceptible to these newer agents[31]. The first vancomycin-resistant *S. aureus* (VRSA) was documented in 1999 and was linked to the acquisition of plasmids or transposons from Enterococcus species encoding the *van* gene cluster, facilitating the addition of D-lactate instead of D-alanine on the peptidoglycan stem peptide[32]. VRSA isolates continue to be very rare[33] and are generally also resistant to telavancin and dalbavancin. However, VRSA isolates remain susceptible to oritavancin[34].

Since 2000, 3 other anti-staphylococcal antibiotics have been developed to treat MRSA: linezolid in 2000, daptomycin in 2003, and ceftaroline in 2010. Linezolid is an oxazolidinone antibiotic that binds to the 23S rRNA, thus inhibiting protein synthesis and growth[35]. Resistance to linezolid is most commonly associated with mutations within the 23S rRNA[36,37]. Resistance to daptomycin, a lipopeptide antibiotic[38], is typically linked to long-term use of the agent to treat recurrent staphylococcal disease[39-42]. Ceftaroline halts peptidoglycan synthesis via binding to PBPs, including PBP2A, and inhibiting their activity[43]. Resistance to ceftaroline is rare, but has been reported in MRSA[44-46].

Lastly, rifampin is an antibiotic that is commonly used to treat staphylococci that are growing within a biofilm on a foreign body. This anti-biofilm activity is hypothesized to be linked to the dependence of quiescent niches within the biofilm on RNA synthesis. Rifampin is a bactericidal antibiotic that inhibits RNA synthesis[47]. However, resistance to rifampin is rapidly selected because of single-point mutations within *rpoB* of both *S. aureus* and *S. epidermidis*[48,49]. Thus, rifampin should not be used as monotherapy.

Cutibacterium acnes

C. acnes (previously known as *Propionibacterium acnes*) is a normal constituent of the human microbiota but has been implicated in a wide variety of maladies including infective endocarditis, acne, and PJIs. *C. acnes* PJIs most commonly involve the shoulder but have also been identified in the hip and knee[12,50-54]. However, resistance of *C. acnes* to antibiotics that would be used to treat a PJI, including penicillin, ceftriaxone, daptomycin, levofloxacin, linezolid, and vancomycin, has not been reported[55].

Enterococcus faecalis *and* Enterococcus faecium

E. faecalis and *E. faecium,* which are common pathogens associated with PJI of the hip and knee (Table I), are intrinsically resistant to many classes of antibiotics, including cephalosporins and aminoglycosides[55]. However, although both species are intrinsically resistant to cephalosporins, *E. faecalis* is highly susceptible to penicillin[55,56]. In contrast, most clinical strains of *E. faecium* are resistant to penicillin

TABLE I The Increase of Resistant Gram-Positive Bacteria Expressed Over Time*

Study Period and Study†	MSSA	MRSA	MSSE†	MRSE§	Streptococcus	Enterococcus	*C. acnes*	Polymicrobial	Mycobacteria and Fungi	Total No. of Infections#
Before 1990										
McDonald[118] (1989)	19	NR	37	NR	19	NR				102
Berbari[119] (1998)	101	NR	86	NR	42	6		88	4	451
Tsaras[5] (2012)	19	2	18	NR	13	3		8	1	72
Windsor[120] (1990)	4	NR	8	NR	4			6		45
Inman[121] (1984)	19	NR	40	NR	11	8		6	NR	98
Segreti[6] (1998)	5	2	3	3	2			2		18
Total	167	4	192	3	91	17	0	110	5	
1990 to 1999										
Fitzgerald[122] (1995)	19	NR	30		14	5			5	102
Toulson[123] (2009)	23	7	18	12	6	5			1	85
Volin[124] (2004)	8	2	14	7	3	4				46
Garvin[125] (1993)	18	NR	36	NR	10	4				98
Marculescu[86] (2006)	30	2	23	NR	14	3		8	1	97
Kilgus[7] (2002)	10	17	9	12	NR	NR	NR	12	1	65
Total	108	28	130	31	47	21	0	20	8	
2000 to 2009										
Berend[126] (2013)**	63	37	NR		17			19		165
Bjerke-Kroll[50] (2014)	145	34	112	41	86	41	3	NR	5	1,080
Aggarwal[10] (2014)††	239	114	154	79	48	30	NR	57	18	919
Pulido[127] (2008)	12	12	6	7	8	NR	NR	4		63
Kusuma[128] (2011)	15	9	12	16	3	2				76
Shukla[129] (2010)	20	13	16	13	8	3				91
Total	494	219	300	156	170	76	3	80	23	
2010 to present										
Klement[12] (2019)	110	56	54	NR	53	5	5	29	6	189
Hartman[13] (2022)	33	10	50	NR	19	12		19	2	170
Tai[130] (2022)††	497	NR	108	NR	287	155	164	508	77	2391
Total	143§§	66	212	0	359	172	169	556	85	

*NR = not reported, MSSA = methicillin-susceptible *Staphylococcus aureus,* and MSSE = methicillin-susceptible *Staphylococcus epidermidis*. †Studies that showed resistant bacteria are listed chronologically by the mean year of surgery. ‡This category included MSSE and other methicillin-susceptible coagulase-negative staphylococci. §This category included MRSE and other methicillin-resistant coagulase-negative staphylococci. #The total number of infections includes infections with anaerobes and gram-negative organisms as well as all culture-negative infections, which are not listed in the table. **Pathogens were grouped as susceptible or resistant; species were not identified. ††Only the U.S. data for this study were included; the numbers were calculated from the total and, of the reported percentages, 49.6% of the staphylococci were resistant. ‡‡Tai et al. added *Staphylococcus lugdunensis* to the coagulase-negative staphylococci; resistance was not reported. §§These totals do not include the study by Tai et al., as resistant pathogens were not reported in their study.

and ampicillin[55,56]. Similar to the treatment of MRSA, daptomycin and linezolid are mainstays for the treatment of VRE.

Viridans Group Streptococcus

Similar to *Streptococcus pneumoniae,* Viridans group Streptococcus is commonly resistant to β-lactam antibiotics including penicillin, ceftriaxone, and meropenem[57,58]. Resistance is not due to production of β-lactamases; instead, it is due to natural transformation involving the acquisition of genes for penicillin-binding proteins that do not bind β-lactam antibiotics[59,60]. Thus, clinical utilization of β-lactam antibiotics requires laboratory testing, as it is difficult to predict susceptibility. Many clinicians may use oral fluoroquinolone therapy to manage patients with Viridans group Streptococcus infections, and it is similarly critical to perform susceptibility testing because resistance to ciprofloxacin and levofloxacin is not uncommon[61].

Diagnosis of and Clinical Evidence for Resistance

The diagnosis of a PJI can be challenging, especially when patients have been administered antibiotics before a culture specimen has been obtained, when the culture is not allowed adequate incubation time, or when fungi or atypical pathogens are not assessed with appropriate cultures. The criteria and tests used to diagnose PJI are ever-evolving. The Musculoskeletal Infection Society (MSIS) has developed and subsequently modified diagnostic criteria[62,63]. However, the use of culture to diagnose PJI is generally insensitive and does not yield an offending pathogen in up to ≥30% of infections[64-66]. To aid the clinician in identifying the presence of infections, newer diagnostics with high accuracy have been developed including those that detect alpha defensin, interleukin [IL]-6, and neutrophil elastase[67]. Because of the limitations of cultures, molecular methods of identification have gained traction over the past decade. Most recently, the BioFire Bone and Joint Infection Panel has been approved by the U.S. Food and Drug Administration (FDA) for use in the detection of 31 different bacterial and yeast targets and 8 different resistance markers from synovial fluid[68]. Unfortunately, this panel cannot detect 2 common PJI pathogens, *S. epidermidis* and *C. acnes.* Next-generation sequencing (NGS) using 16S eubacterial primers and 18S fungal primers has also aided in the detection of pathogens in culture-negative cases of PJI, and it can include the detection of antibiotic resistance[69]. Further methods utilizing shotgun and targeted NGS approaches have accelerated pathogen discovery from synovial fluid specimens. The sensitivity utilizing this approach has ranged from 61% to 94%, and specificity has ranged from 73% to 100%[69-71]. This technology has the potential to not only identify resistance but also identify new resistance patterns more quickly. Street et al. revealed improved antimicrobial-resistance detection using specific metagenomic sequencing techniques, with a sensitivity of 87%[72]. Furthermore, NGS results can be available within 48 hours, compared with 14 days for cultures. NGS does have limitations, including data interpretation issues and DNA contamination due to normal flora, which may lead to confusion with regard to appropriate treatment[69,71]. Lastly, another method in the detection of culture-negative PJI is the sequencing of circulating cell-free microbial DNA from peripheral blood. This approach has been utilized to identify pathogens causing PJIs[73].

Clinical Evidence of Resistance

Confirmation of the emergence of resistant bacteria in PJI is difficult to trace in the literature. Articles published before the 1990s rarely mentioned the type of bacteria or any resistance patterns (Table I). Typically, if the bacteria were identified, it was by their genus and species, not their susceptibility to antibiotics. One of first articles comparing resistant and sensitive pathogens found that a higher percentage of patients with a periprosthetic hip or knee joint infection with antibiotic-sensitive bacteria were successfully treated compared with those with antibiotic-resistant bacteria[7]. Articles that have reported bacteria associated with PJI including documentation of resistance patterns are listed in Table I.

Treatment of Resistant Pathogens

The management of infections due to drug-resistant pathogens is complex, and there is a paucity of antibiotics in development, some of which may only provide a limited benefit over current agents[74,75] (Fig. 1). Newer antimicrobial agents with novel modes of action are needed to combat the ongoing development of resistance, and funding antibiotic development efforts is of utmost importance so that we can continue to provide effective therapeutic options for our patients[76,77].

Surgical Management of Resistant Pathogens

Management of PJI caused by resistant pathogens has not changed drastically over the past decades. In general, acute infections are managed with debridement, antibiotics, and implant retention (DAIR) with modular component exchange. Chronic infections are managed with either 1 or 2-stage exchange. Although surgical management is not necessarily altered when managing resistant pathogens, organism identification remains critical to the successful treatment of PJI. Many studies have shown that success rates can vary widely on the basis of the offending organism[78-82].

DAIR Outcomes

DAIR is typically indicated in patients with acute perioperative or hematogenous PJI. However, this technique can be performed in patients with chronic infections who may not be candidates for 2-stage exchange or who have difficult-to-remove components. Recently, the eradication success for DAIR was reported to be dependent on the infecting organism regardless of the chronicity of infection[83]. Overall, success rates have varied widely from 18% to 87%[84-88]. Treatment with DAIR has a failure rate of 57% in patients infected with *S. aureus*[88] and up to 84% in patients infected with MRSA[84,89]. Evolving techniques such as rapid 2-stage with implant retention[90] and the addition of intraosseous vancomycin[91] have reported eradication rates of 93.8% and 92.3%, respectively. Overall, surgeons should be aware of the indications and outcomes for DAIR and attempt to identify the organism for optimal results.

One and 2-Stage Outcomes

There are no studies to date that have identified the superiority of either 1 or 2-stage approaches, although many authors have cautioned against using a 1-stage technique in the setting of a resistant pathogen[92,93]. We are aware of only 1 report using 1-stage exchange in the setting of resistant organisms. Ohlmeier et al. reported an infection control rate of 93.1% using this technique for MRSA infections in 29 patients at the ENDO-Klinik[94]. Other authors have reported failure rates between 21% and 35% using 2-stage exchange in patients with MRSA or MRSE[95,96], which represents a twofold to fourfold increased risk of treatment failure compared with nonresistant infections[78,96]. Only 1 study showed antibiotic resistance developing between the 2 procedures, at a rate of 7.04%. Those authors considered this rate to be relatively low and thus indicating the safety of prolonged use of antibiotics during this treatment option[97]. Because PJI treatment in the setting of resistant organisms

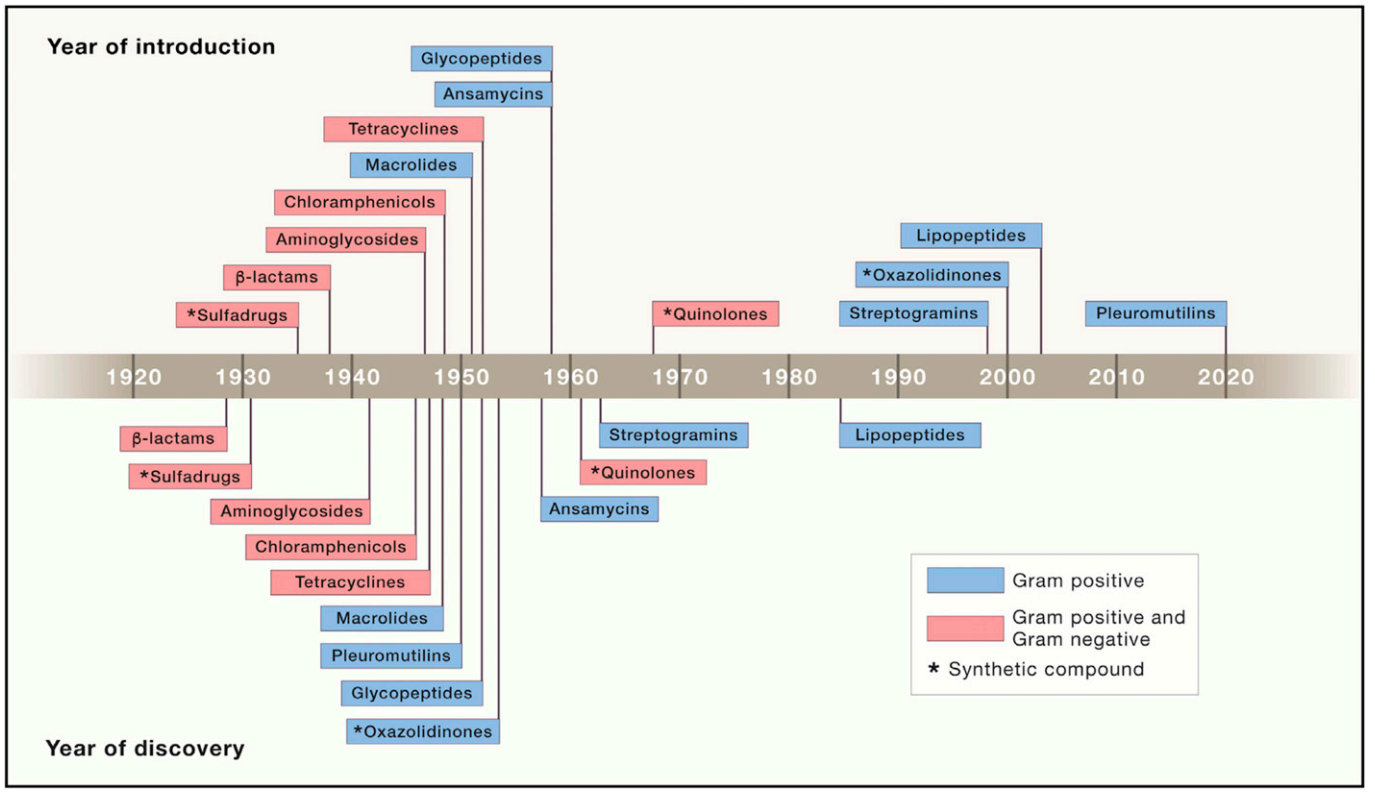

Fig. 1

The timeline of antibiotic discovery. *Bottom:* Year of discovery. *Top:* Year when the first member of the class was introduced into clinical practice. Broad-spectrum antibiotics are shown in red. (Reprinted from Cell, 2020 Apr 2;181[1], Lewis K, The science of antibiotic discovery, p 29-45, Copyright 2020, with permission from Elsevier.)

continues to be a challenge, research should focus on the safety and effectiveness of all potential surgical approaches. Although 1-stage exchange has obvious advantages related to the timeline for restoring patient function and potentially enhancing antibiotic stewardship, limited data exist to define an optimal surgical approach.

Antimicrobial Agents with Activity Against Drug-Resistant, Gram-Positive Pathogens

Multiple antimicrobial agents have been utilized for the management of multidrug-resistant, gram-positive pathogens (Table II). It should be noted that, although these antimicrobial agents are often used in clinical practice for complicated bone and joint infections, supporting evidence in the medical literature is scant. Multiple factors with regard to the pathogen, the clinical scenario, and the characteristics of the antimicrobial agent should be considered when choosing antimicrobial therapy for complicated bone and joint infections. Given the complexity of the management of complicated bone and joint infections with multidrug-resistant organisms, patients should be managed collaboratively in consultation with an infectious diseases specialist, when available, to direct the antimicrobial therapy course, in order to deliver the best care for the patient using a multidisciplinary approach.

Combination Therapy

The most common combination therapy utilized in PJI involves the use of adjunctive oral rifampin along with another active antimicrobial agent. Rifampin is a potent anti-staphylococcal drug with the ability to penetrate biofilm. There are studies that have suggested a benefit of adding adjunctive rifampin to fluoroquinolone therapy for the management of PJI[98-100]. However, other studies involving non-fluoroquinolone combinations demonstrated no evidence of benefit. A recent systematic review and meta-analysis demonstrated a 10% increase in success rate when rifampin was used as part of a combination therapy regimen for staphylococcal PJI after DAIR; however, the vast majority of the included studies were observational and encumbered by multiple biases[101]. Based on the available data, the addition of adjunctive rifampin should be considered in certain clinical scenarios, especially as part of a combination therapy regimen with a fluoroquinolone. Rifampin should not be given as monotherapy because resistance tends to emerge quickly. It is also a potent inducer of the cytochrome p450 system, which may result in important drug-drug interactions, including with common anticoagulants as well as many other medications.

The role of combinations of vancomycin or daptomycin with β-lactam antibiotics in the management of severe MRSA infections has been proposed because of the potential for

TABLE II Antimicrobial Agents Used to Treat Infections Due to Multidrug-Resistant, Gram-Positive Organisms*

Drug	Year Approved	Class	Route of Administration	Mechanism of Action	In Vitro Gram-Positive Activity	Comments
Vancomycin	1958	Glycopeptide	Intravenous (oral formulation only used to treat *Clostridoides difficile* due to lack of systemic absorption)	Inhibits cell wall synthesis	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (non-VRE)	Requires monitoring of therapeutic drug levels; MIC for gram-positive pathogens is increasing; some enterococci have developed resistance (VRE); nephrotoxicity and infusion reactions can occur
Quinupristin-dalfopristin	1999	Streptogramin	Intravenous	Inhibits protein synthesis	MSSA, MRSA, coagulase-negative staphylococci, streptococci, *E. faecium*	Severe arthralgias and myalgias resulted in limited use
Linezolid	2000	Oxazolidinone	Intravenous, oral	Inhibits protein synthesis (50S ribosomal subunit)	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including VRE)	Highly bioavailable; myelosuppression, neuropathy, serotonin syndrome, and lactic acidosis can occur, and risk increases with duration of use
Daptomycin	2003	Lipopeptide	Intravenous	Cell membrane disruption	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including VRE)	Emergence of resistance has been described, particularly in enterococci, including during the initial course of therapy; monitoring for rhabdomyolysis (by creatine kinase) is important for doses > 6 mg/kg per day; eosinophilic pneumonia has been reported
Tigecycline	2005	Glycylcycline	Intravenous	Inhibits protein synthesis (30S ribosomal subunit)	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including VRE)	Broad-spectrum activity; pharmacodynamic properties limit use to specific indications or combination therapy; increased risk of all-cause mortality in patients receiving tigecycline relative to comparator agents. Gastrointestinal side effects in up to one-third of patients.
Telavancin	2009	Lipoglycopeptide	Intravenous	Inhibits cell wall synthesis	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including *vanB* VRE only)	Derivative of vancomycin, fixed once-daily dosing. Can cause mild QT prolongation.
Ceftaroline	2010	Cephalosporin	Intravenous	Inhibits cell wall synthesis, binds to penicillin-binding protein (including PBP2A)	MSSA, MRSA, coagulase-negative staphylococci, streptococci, *Enterococcus faecalis* (non-VRE)	Fifth-generation cephalosporin; broad-spectrum activity, including some VISA; neutropenia common with extended courses

continued

TABLE II (*continued*)

Drug	Year Approved	Class	Route of Administration	Mechanism of Action	In Vitro Gram-Positive Activity	Comments
Dalbavancin	2014	Lipoglycopeptide	Intravenous	Inhibits cell wall synthesis	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including *vanB* VRE only)	Analog of vancomycin, prolonged half-life. Infusion reactions can occur, most commonly with rapid (<30 minutes) infusions.
Oritavancin	2014	Lipoglycopeptide	Intravenous	Inhibits cell wall synthesis	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including VRE)	Analog of vancomycin, prolonged half-life
Tedizolid	2014	Oxazolidinone	Intravenous, oral	Inhibits protein synthesis (50S ribosomal subunit)	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including VRE)	Highly bioavailable; once-daily dosing and more favorable side effect profile compared with linezolid; myelosuppression and neuropathy can occur
Delafloxacin	2017	Fluoroquinolone	Intravenous, oral	Blocks DNA replication	MSSA, MRSA, coagulase-negative staphylococci, streptococci, *E. faecalis* (non-VRE)	Broad-spectrum activity; good bioavailability; tendinopathy and tendon rupture, peripheral neuropathy, and CNS effects can occur
Omadacycline	2018	Tetracycline	Intravenous, oral	Inhibits protein synthesis (30S ribosomal subunit)	MSSA, MRSA, coagulase-negative staphylococci, streptococci, enterococci (including VRE)	Broad-spectrum activity; unique chemical structure allows it to overcome other tetracycline resistance mechanisms; gastrointestinal side effects are relatively common

*It is important to note that this list is not exhaustive, as other agents may be employed for the management of infections due to gram-positive multidrug-resistant organisms on a case-by-case basis depending on the susceptibility pattern of the microorganism. MSSA = methicillin-sensitive *Staphylococcus aureus*, MIC = minimum inhibitory concentration, *vanB* and *vanA* = vancomycin resistance genes, and CNS = central nervous system.

synergistic activity[102]. Ceftaroline has also been used off-label as part of a combination therapy regimen for MRSA bloodstream infections. These combination therapies are typically used following the failure of conventional regimens for persistent MRSA bacteremia. These complicated antimicrobial regimens should only be used after careful consideration, with expert guidance and close monitoring.

Potential Adjuvant Therapies

Although systemic antimicrobial therapy remains the mainstay of conventional PJI treatment, other strategies have been developed to supplement and enhance systemic therapy. Bacteriophages are viruses that specifically target and infect bacterial cells and have demonstrated success in the management of a variety of bone and joint infections in preclinical studies, case reports, and a few small case series[103]. In these studies and reports, bacteriophages were utilized in the management of patients with infections due to multidrug-resistant organisms, as well as in relapsing infections[104,105]. Several studies seeking to evaluate the safety, tolerability, and treatment success of bacteriophage therapy in patients with PJI are planned or underway[106].

A variety of therapeutic modalities targeting modulation of the host immune response, novel local antibiotic delivery mechanisms, and the use of nanoparticles and antimicrobial peptides to aid in the management of patients with implant-related bone and joint infections are all in various stages of investigation[107,108].

Preventing the Emergence of Bacterial Resistance to Antibiotics

The overuse and misuse of antibiotics have led to substantial antimicrobial resistance, so there is particular interest in

TABLE III Antibiotic Stewardship Controversy in Total Joint Arthroplasty*

Topic†	Study	Level of Evidence	No. of Patients	Study Design	Follow-up	Results and Conclusions
Perioperative antibiotics						
1 dose vs. multiple doses	Ryan[131] (2019)	I	9,691	Meta-analysis	—	No difference
	Wymenga[132] (1991)	I	3,013	RCT	—	No difference, but limited number of patients prevented significance
	Siddiqi[133] (2019)	III	51,627	Meta-analysis	—	No difference
Extended oral antibiotics	Inabathula[134] (2018)	III	2,181	Retrospective	90 days	Significant infection reduction in high-risk population: RR, 4.0 for THA ($p = 0.037$) and 4.9 for TKA ($p = 0.009$)
	Kheir[135] (2021)	III	3,855	Retrospective	1 year	Significant infection reduction in high-risk population
	Carender[136] (2021)	Not defined	650	Retrospective	90 days	No difference in BMI > 40 kg/m^2
	Zingg[137] (2022)	Not defined	176	Retrospective	3 years	2.2% risk of infection with 7-day oral antibiotic after aseptic TKA revision
Intraoperative antibiotics						
Antibiotic-loaded bone cement	Bendich[138] (2020)	III	15,972	Retrospective	5 years	Lower rate of PJI revision with antibiotic-loaded bone cement
	Jameson[139] (2019)	Not defined	731,214	Registry	10 years	Significant reduction in revision with antibiotic-loaded bone cement
	Tayton[140] (2016)	Not defined	64,566	Registry	10 years	Increased odds of infection with antibiotic-loaded bone cement
Antibiotic powder	Peng[141] (2021)	III	4,512	Meta-analysis	—	Decreased risk of surgical site infection
	Iorio[142] (2020)	IV	3,251	Retrospective	90 days	May reduce risk of PJI in high-risk population
	Buchalter[143] (2021)	Not defined	12,066	Retrospective database	90 days	Reduced early PJI risk
Prophylactic antibiotics						
Oral antibiotics after PJI treatment	Frank[144] (2017)	I	107	RCT	14 months	5% vs. 19% failure rate secondary to infection with 3-month oral suppression after 2-stage exchange ($p = 0.016$)
	Johnson[145] (2013)	Not defined	66	Retrospective	2 years	Infection rate: 0% in those receiving antibiotics, 13.6% in those receiving no antibiotics, and 0.5% in those undergoing aseptic revisions
Lifetime suppression	Siqueira[146] (2015)	IV	655	Retrospective	5 years	68.5% vs. 41.1% ($p = 0.008$) infection-free survival after 2-stage and DAIR
	Bryan[87] (2017)	Not defined	90	Retrospective	6 years	After DAIR, reinfection rate of 3% for those on lifetime suppression vs. 11% not on suppression
	Weston[147] (2018)	Not defined	134	Retrospective	5 years	66% infection-free survival after DAIR
Dental	Quinn[148] (2017)	Not defined	Appropriate use criteria		—	Recommended dental prophylaxis in certain clinical scenarios
	Sollecito[149] (2015)	Not defined	Clinical practice guidelines		—	Did not recommend dental prophylaxis

*RCT = randomized controlled trial. †Controversial topics regarding antibiotic stewardship.

TABLE IV Grades of Recommendation Regarding Resistant Bacteria

Recommendations for Care	Grade*
Careful patient selection and improving or correcting risk factors for PJI before elective surgical treatment are strongly recommended.	A
Appropriate microbiological methods, including those used to detect and grow *C. acnes,* are recommended.	A
Antimicrobial agents used in the prevention or management of infection should be selected appropriately and the duration of therapy carefully considered in order to mitigate the risk of development of bacterial resistance.	B
Molecular methods including rapid PCR diagnostics, 16S sequencing, and/or shotgun and/or targeted whole-genome sequencing are recommended in culture-negative cases of PJI.	I
Expert consultation with an infectious diseases specialist (if available) is recommended to assist with the appropriate antimicrobial management and monitoring of patients with PJI.	I

*According to Wright[150], grade A indicates good evidence (Level-I studies with consistent findings) for or against recommending intervention; grade B, fair evidence (Level-II or III studies with consistent findings) for or against recommending intervention; grade C, poor-quality evidence (Level-IV or V studies with consistent findings) for or against recommending intervention; and grade I, insufficient or conflicting evidence not allowing a recommendation for or against intervention.

modalities to slow or stop the development of resistance[10,89]. Two industries that involve the use of antibiotics are food production and medicine. The use of broad-spectrum antibiotics in these industries creates an environment that selects for resistance genes that can be readily transferred. Antimicrobial use in the food chain for animal production is arguably one of the greatest factors contributing to antimicrobial resistance. Although many of the world's top meat-producing countries have banned the use of antibiotics as growth promoters in livestock, countries such as the People's Republic of China, Russia, and India still allow farmers to use antibiotics as growth promoters in livestock[109]. One of the first reports of antimicrobial use and its effect on antibiotic resistance involved avoparcin-resistant enterococci. Avoparcin, a glycopeptide antibiotic, was used as a food additive to promote the growth of animals. Shortly after its use in animal feed, VRE were detected[110,111]. Avoparcin was soon removed by the European Union, emphasizing how serious a public health problem antimicrobials can be when placed in animal feed.

Studies have shown that a large proportion of antimicrobial use in health care is inappropriate[112]. Antimicrobial stewardship is a systematic approach to the use of antimicrobial agents to achieve optimal outcomes. This approach involves ensuring that the correct antimicrobial agent is utilized at the correct dose for the appropriate duration in order to effectively treat infections while minimizing toxicity and emergence of resistance[113]. Preauthorization and prospective audit and feedback antimicrobial stewardship programs have been shown to reduce inappropriate antimicrobial use in multiple care settings, which translates into reductions in antibiotic resistance and hospital-acquired infections as well as cost savings[114]. There remains much debate around antibiotic stewardship in adult reconstructive surgery, and there is a lack of high-quality studies to support identification of the best practice. These controversies are best displayed in Table III.

Aside from antibiotic stewardship programs, patient selection in TJA also has a role in preventing PJI. Reducing the number of surgical procedures performed on patients with identifiable risk factors will theoretically reduce the PJI burden of sensitive and resistant pathogens. Many studies to date have identified independent patient risk factors for PJI. These data introduce a dilemma regarding whether or not to treat patients with debilitating end-stage arthritis. Kunutsor et al. identified male gender (relative risk [RR], 1.36 [95% confidence interval (CI), 1.18 to 1.57]), smoking (RR, 1.83 [95% CI, 1.24 to 2.70]), body mass index (BMI) > 40 kg/m^2 (RR, 3.68 [95% CI, 2.25 to 6.01]), diabetes (RR, 1.74 [95% CI, 1.45 to 2.09]), rheumatoid arthritis (RR, 1.70 [95% CI, 1.37 to 2.11]), depression (RR, 1.48 [95% CI, 1.13 to 1.95]), and previous joint surgery (RR, 2.98 [95% CI, 1.49 to 5.93]) as significant risk factors for PJI in a large systematic review and meta-analysis including 66 studies and >500,000 patients[115]. Evidence does support decreased risk of infection in patients who optimize modifiable risk factors prior to undergoing a surgical procedure[116,117].

Overview

In conclusion, a documented increase in resistant bacterial pathogens has been observed over the last 4 decades. Recommendations to better understand and manage resistant bacteria are provided (Table IV). A principal way to lower the risk of PJI caused by resistant pathogens is to perform a careful preoperative evaluation, including correcting modifiable risk factors for PJI before elective surgical treatment. If an infection does occur, utilization of appropriate microbiological methods is recommended. In cases of culture-negative PJI, molecular methods including rapid polymerase chain reaction (PCR) diagnostics, 16S sequencing, and/or shotgun and/or targeted whole-genome sequencing are recommended; because we do not have an absolute test to use as a baseline, we can only compare their results with those of other clinical diagnostic parameters that we currently use in practice. Antimicrobial agents used in the prevention or management of infection should always be selected appropriately, and the duration of therapy should be carefully considered to mitigate the risk of

the development of bacterial resistance during and after therapy. Finally, expert consultation with an infectious diseases specialist (if available) is strongly recommended to assist with the appropriate antimicrobial management and monitoring of patients with PJI. ■

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Current Concepts Review

Application of Nucleic Acid-Based Strategies to Detect Infectious Pathogens in Orthopaedic Implant-Related Infection

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- Implant-associated infection in orthopaedic surgery remains an enormous and largely unsolved clinical problem with a high rate of persistent or recurrent infection. This may be due, at least in part, to the potential for underdiagnosis by traditional microbial culture or the potential for culture to incompletely identify the microbial species present.
- Nucleic acid-based diagnostic techniques, focused on using the diagnostic information contained in DNA or RNA to identify microbial species, have been developing rapidly and have garnered escalating interest for both clinical and research applications.
- Commonly applied techniques include end-point polymerase chain reaction (PCR), quantitative PCR, Sanger sequencing, and next-generation sequencing. Understanding the specific strengths and weaknesses of each technique is critical to understanding their utility, applying the correct assessment strategy, and critically understanding and interpreting research.
- The best practices for interpreting nucleic acid-based diagnostic techniques include considering positive and negative controls, reads per sample, detection thresholds (for differentiating contaminants from positive results), and the primer set or targeted regions.

Implant-associated infection in orthopaedics remains a largely unsolved clinical problem with unacceptably high rates of treatment failure requiring reoperation, with rates exceeding 30%[1-3]. The consequences are devastating, with risk of recurrence, chronic dysfunction, amputation, and death in both trauma and arthroplasty populations[4-11]. Current treatment strategies focus on systemic antibiotics targeted against pathogens identified via culturing in association with surgical debridement and removal of implants. However, this treatment strategy has an unacceptably high rate of failure, which is likely due, at least in part, to issues with traditional culturing methods that may miss clinically relevant microbial species. Culture-negative infection and/or infection with incomplete identification of infecting species may result in inadequate antibiotic coverage, which very likely contributes to recurrence. This is clearly reflected in the inferior outcomes and higher recurrence rates associated with culture-negative infection compared with infections with identified microbial species[12].

Microbiological culture-based strategies have serious limitations, despite their status as the gold standard. Culture yields negative results in 7% to 50% of periprosthetic joint infection cases[13-16] and 30% of fracture-related infection cases[2,17], and there is concern that culture yields, even when positive, may be incomplete. This is related to several issues. First, traditional culturing methods are biased toward organisms that thrive under nutritional, atmospheric, and physiological conditions employed by diagnostic laboratories (common culture challenges reviewed by Lewis et al.[18]), which are different from physiological conditions that exist in implant-associated infection. Several studies have demonstrated that culture results insufficiently represent

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the entirety of bacterial communities in infected wounds[19-21]. Second, traditional culture is biased toward planktonic free-floating microbes, compared with biofilm-based microbial communities. This likely results in a culture yield that misses the most important species for infection recurrence[19-22]. Third, some microbes flourish only when a second species is also present (polymicrobial cultures)[23-28]. For obligately polymicrobial infections, traditional culturing methods may fail to isolate causative pathogens. Fourth, culture is associated with a 3 to 10-day delay until the identification of the species. Lastly, there is no quantitative information with regard to the relative bioburden and spatial arrangement of microbial species alone and in combination.

Based on these issues, culture-independent molecular diagnostic techniques have been developing rapidly and have garnered escalating interest for both clinical and research applications[29,30]. A subset of molecular diagnostic techniques focus on diagnostic information contained in nucleic acids (NAs) (Table I). The benefits arising from the sensitivity of NA-based strategies may be tempered by the errors resulting from improperly handling specimens or interpreting data. As NA-based diagnostic techniques become more mainstream, it is critical for orthopaedic surgeons to become facile with the nuances associated with these diagnostic tools. Therefore, in this review, we aim to provide a comprehensive overview of NA-based analysis strategies and review important caveats and best practices around applying or interpreting NA sequencing-based techniques.

NA-Based Analysis Techniques

NA-based microbial assessment strategies, based on deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), may fill the gap between characterizing the most abundant microorganisms and the most clinically relevant microorganisms. Understanding the steps in gene expression is critical to understanding sequencing-based technology. Cells replicate DNA by separating successive small regions of the DNA into 2 single strands. A polymerase reads the single-stranded DNA and adds paired bases to prepare 2 identical double-stranded DNA molecules. Active cells transcribe DNA into RNA in a similar manner, but, instead of copying the entire sequence, they transcribe only a targeted region, resulting in a short strand of single-stranded RNA. Ribosomes bind the resulting messenger RNA (mRNA) and translate its sequence into amino acids to create a protein. Because the function of ribosomes is so essential, their binding abilities are highly conserved across all living organisms. Ribosomal RNA (rRNA) consists of highly conserved binding sites interspersed with hypervariable regions, which can

TABLE I Comparison of Molecular Strategies and Their Applications*

General Technology	Chemistry	Quantitative	Multiplex	Output	Other Names
End-point PCR	PCR			Amplicon	UMD-Universal PCR, rapid ribosequencing
	PMA PCR			Amplicon	
	ddPCR (Bio-Rad Laboratories)	X	X	Amplicon	
	PCR-DGGE		X	Amplicon	
	RFLP		X	Amplicon	
	ESI-MS		X	Amplicon	
qPCR and RT-PCR	DNA-binding dyes	X		Amplicon	
	TaqMan (Thermo Fisher Scientific)	X	X	Amplicon	
	FRET	X	X	Amplicon	
	Molecular beacon	X	X	Amplicon	
	Hybridization probe	X	X	Amplicon	
	MGB Eclipse probe (IDT)	X	X	Amplicon	
	Amplifluor (Sigma-Aldrich)	X	X	Amplicon	
	Scorpion primer (Millipore Sigma)	X	X	Amplicon	
	LUX primer (Invitrogen)	X	X	Amplicon	
	BD QZyme (BD Biosciences)	X	X	Amplicon	
Sanger sequencing	Chain termination			Single sequence	
NGS	Various	X	X	Multiple sequences	Deep sequencing, high-throughput sequencing

*PMA = propidium monoazide, dd = droplet digital, DGGE = denaturing gradient gel electrophoresis, RFLP = restriction fragment length polymorphism, ESI-MS = electrospray ionization mass spectrometry, and FRET = fluorescence resonance energy transfer.

be used to identify organisms at various taxonomic levels[31]. The 16S rRNA gene is a prokaryote-specific sequence that encodes the rRNA component of the ribosome. Sequencing the hypervariable regions of the 16S rRNA gene in DNA allows the identification of bacterial DNA[32]. There are several techniques that take advantage of these processes to identify pathogens, and each has unique advantages and disadvantages (Table II).

NA Extraction

Extraction methods are designed to separate NAs from other materials in a sample (cell debris, proteins, lipids). Extraction protocols begin with cell lysis to release NAs into solution. Subsequent steps include protein precipitation, lipid separation, and salt removal to produce a sample containing concentrated NAs with minimal impurities[33].

End-Point Polymerase Chain Reaction (PCR)

PCR is a technique of amplifying DNA outside the cell[34]. The basic PCR technique requires template DNA, primers, free nucleotides, and DNA polymerase. The reaction mix is heated to melt double-stranded DNA into 2 single strands. The mix is then cooled to allow annealing of primers to targeted sites. Primer sets are designed to include a forward and a reverse

TABLE II NA-Based Analysis Techniques: Advantages and Disadvantages

Technique	Basic Principle	Advantages	Disadvantages
End-point PCR	Uses primers to identify bacterial species qualitatively (not quantitatively)	Qualitative assessment of bacteria Probe for presence of specific taxa or genes (such as methicillin resistance) Low cost Rapid (<12 hr)	Not quantitative Limited by requirement for primer specificity Multiplexing is difficult
qPCR	Similar to end-point PCR, except reaction is monitored continuously to quantify the abundance of gene of interest	Quantitative analysis is possible Multiplexed (or parallel) methods reduce time and reagents required Rapid (<12 hr) Probe for presence of specific taxa or genes	Only targeted genes (amplicons) will be identified Characterization of community variation is not possible Multiplexed reactions are limited to primer sets that require similar reaction conditions
Sanger sequencing	Provides nucleotide sequence of amplicons from pure sample	Inexpensive Rapid (~24 hr) Useful for identifying cultured bacteria	Requires pure monoculture as input, so is susceptible to the same issues as traditional culturing
RNA sequencing	Same as DNA-based technique after an initial step reverse-transcribing cDNA from RNA	Informs which genetic elements are being actively transcribed, indicating biological activity Can inform bacterial viability and host response Speed similar to DNA-based techniques after ~2-hr reverse transcription step	RNA has increased sensitivity to degradation Slow (days to weeks)
NGS	Massively parallel sequencing of NAs; most commonly all variants of the 16S rRNA gene in a sample are sequenced to determine microbial species abundance	Can identify taxa in polymicrobial samples Inexpensive if many samples are run together Allows community analysis of all variants	More expensive and time-intensive than qPCR or Sanger sequencing Increased probability that background or contamination will be amplified Sensitive to contamination Database limitations Slow (4 days to 6 weeks)
Metagenomic NGS	Uses random primers to comprehensively amplify all fragments of NA sequences in a sample	Can generate information about all genes present in sample (such as identification of microbial species as well as virulence and resistance genes)	More expensive than 16S rRNA NGS Additional information can be more difficult to interpret Database limitations Slow (4 days to 6 weeks)

primer that bind to either side of the region of interest. DNA polymerase recognizes regions where primers have annealed and amplifies the DNA to create double-stranded DNA. This is repeated ≥30 times, with the DNA concentration doubling after every cycle. Once enough of the double-stranded DNA amplicon (or product of amplification events) has been produced, it can be visualized by running it on a gel (Fig. 1). Because the product of this reaction is only observed at the end of all cycles, this technique is called end-point PCR (Table I). It has been applied in studies of musculoskeletal infection and sepsis (Table II; see also Appendix Supplemental Table 1)[35,36].

Quantitative PCR (qPCR)

The qPCR methods are based on principles that are identical to those of end-point PCR[37]. However, instead of amplicon detection only at the end of all cycles, the reaction is monitored continuously at each cycle to quantitatively determine the amount of the gene of interest in the sample (Fig. 1). This also has been applied to musculoskeletal infection (see Appendix Supplemental Table 1)[38,39]. In multiplex qPCR, several PCR reactions for specific targets are performed in the same reaction mix. Results are teased apart due to differing amplicon length or release of fluorescent label upon successful amplification (Tables I and II).

Sanger Sequencing

In Sanger sequencing (Table I), the sequence of an amplicon is deduced by determining the identity of the base at each position over the amplicon length (Fig. 1)[40]. This is accomplished by including terminating nucleotides in the reaction mix, which prevent the PCR from proceeding. By measuring the length of the amplicon and knowing the identity of the succession of terminating nucleotides at each step, the identity of the base at each position can be inferred. Modern technology has allowed incorporation of fluorescent labels, instead of radioactively labeled nucleotides, that can be run on a flow cytometer and read automatically.

Sanger sequencing of the 16S rRNA gene can determine the probable identity of bacteria by comparing the determined sequence with a database of known 16S rRNA gene sequences. However, this can only be done on monocultures. Sequencing a polyclonal or impure culture results in unusable sequences. Amplification occurs, but there is too much ambiguity in the base at each position for identification. Monocultures must be grown from the specimen prior to Sanger sequencing. If the most relevant microorganism is slower-growing than others, the microbiology laboratory may only identify the first colonies that grow on plates and dispose of cultures before slower-growing strains are visible (Table II).

RNA Sequencing

All of the NA-based techniques described above use DNA. If an initial step of reverse-transcribing complementary DNA (cDNA) from RNA is added, the same techniques can detect RNA in a sample (reverse-transcription PCR [RT-PCR]).

Next-Generation Sequencing (NGS)

NGS involves massively parallel sequencing of the NAs present within a sample. PCR-generated amplicons may be separated by physical methods (i.e., binding to a chip surface) or through dilution (i.e., capillary electrophoresis). The separated amplicons are then monitored and sequenced in parallel. NGS methods include nanopore sequencing (bases identified by measuring charge fluctuation as single-stranded DNA passes through a nanopore[41]), sequencing by synthesis (modern versions of Sanger sequencing in which fluorescent labels on terminating nucleotides are removed, allowing the process to continue, after observation), and sequencing by ligation (similar to Sanger sequencing except that bases are added in 3-mers or 4-mers instead of individually) (Table III)[42,43].

Single-amplicon NGS sequences all variants of a single amplicon in a single sample. This is commonly used for microbial community taxonomic composition analysis by sequencing the 16S rRNA gene (occasionally called 16S rRNA sequencing)[32]. Metagenomic NGS uses random primers to comprehensively amplify all fragments of NA sequences (the metagenome) in a sample. Random primers are designed to bind to a broad range of genome locations and do not target specific sequences.

Researchers may use ≥2 NA-based techniques in parallel or series[32]. Commonly, analysis is performed using NGS of 16S rRNA gene amplicons, followed by end-point PCR or qPCR to confirm the presence of resistance and/or virulence genes. Resequencing a sample to identify the presence of resistance genes is more rapid than waiting for culture-based antibiotic resistance analysis.

NA-based techniques are multistep processes with multiple points at which contamination (introduction of non-sample-specific NAs) can occur (Fig. 2, Table IV), including initial collection, NA extraction, initial PCR, sequencing, and post-sequencing data processing.

Potential Benefits of Molecular Pathogen Identification Strategies

Molecular diagnostic strategies show real promise in advancing how infection is defined and how pathogens are identified for targeted treatment. Until recently, the definition of infection has been based around positive cultures. However, this excludes culture-negative infections, creating both diagnostic and treatment challenges. These issues have led to the development of diagnostic criteria incorporating the Musculoskeletal Infection Society (MSIS)[44,45] and fracture-related infection[46] consensus definitions. Several biomarkers and clinical findings have been identified to aid in the diagnosis[47-50], and these have been integrated into consensus definitions. However, although these biomarkers help to establish the presence of infection, they do not identify organisms and are, therefore, unable to guide targeted treatment. Furthermore, there appears an indeterminate subset of patients who are not mounting an aggressive inflammatory response (one resulting in signs such as purulence, a sinus tract, elevated biomarkers) who may also have clinically relevant infections, such as in the setting of nonunion or aseptic loosening of prosthetic joints. We anticipate that a thoughtful, data-driven molecular diagnostic approach may inform our overall understanding of what constitutes an infection in a treatment-oriented manner.

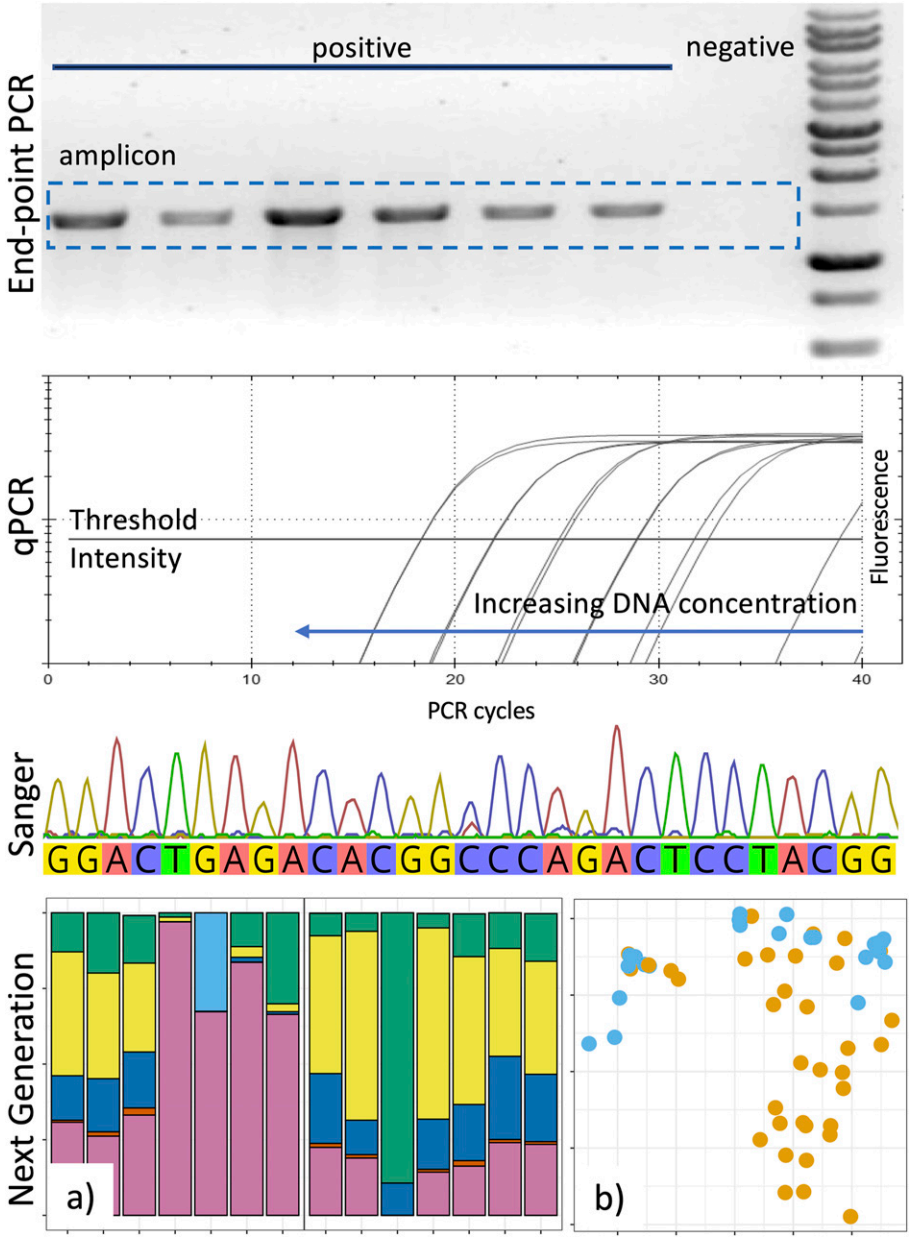

Fig. 1

Typical output of NA-based molecular techniques. In the top image, end-point PCR results are visualized as bands on an agarose gel. DNA fragments (amplicons) travel through the gel based on the number of nucleotides in the sequence (size), with shorter amplicons moving faster. When an amplicon is produced via PCR, a band can be seen. The intensity of the band indicates the concentration of the amplicon in the reaction, but the width of the band is not relevant. Reference ladder(s) containing multiple amplicons of known sizes (far right) are included on the gel for comparison. A positive result is observed as a band on the gel that has traveled the same distance as band(s) in the ladder corresponding to the size of the region of interest. No band (second from right) or a band of the wrong size indicates a negative result. In the second image, qPCR results are visualized in an amplification plot. Fluorophores are released after each successful amplification of the region of interest, resulting in an increase in fluorescence intensity (y axis) as the concentration of DNA increases in the reaction well. The fewer cycles of PCR (x axis) that a reaction must undergo to reach a threshold fluorescence (horizontal bar), the higher the initial concentration of DNA in the sample. In the third image, Sanger sequencing results are visualized as a chromatogram. Terminating fluorophores at each position in the DNA sequence are observed as peaks in fluorescence. At each position in the amplicon, the specific fluorescence (corresponding to 1 of the 4 nucleotides) indicates the base present at that position. NGS results are visualized in many ways. In the bottom image, (a) in stacked bar charts, each bar represents a single sample and each color indicates the proportional abundance of a single taxon inferred to be present in the sample, and (b) similarity in microbial taxonomic composition between samples is often visualized via principal coordinates analysis, where each dot represents a single sample and the 2-dimensional distance between dots indicates the distance between communities.

TABLE III Comparison of NGS Technologies

Chemistry	Other Names	Accuracy (Q30*)	Run Time	Total Output Data Size	Max. Read Length	Max. Reads per Run	Input Required	Max. Samples per Run	Technology Status
Pyrosequencing	Roche 454 GS-FLX Titanium (Roche)	85%	24 hr	0.7 Gb	700 bp	500,000	Not published	Not published	Discontinued
Reversible terminator chemistry	Illumina MiSeq (Illumina)	97%	55 hr	15 Gb	2 × 300 bp	25 million	ng	192	Current
	Illumina HiSeq (Illumina)	95%	2 to 6 days	150 Gb to 1 Tb	2 × 150 bp	2 to 4 billion	ng	384	Discontinued
	Illumina NextSeq (Illumina)	75%	35 hr	90 Gb	2 × 150 bp	400 million	ng	384	Current
	Illumina genome analyzer (Illumina)	98%	3 to 10 days	4 to 25 Gb	2 × 75 bp	300 million	100 ng	12	Current
	Illumina NovaSeq (Illumina)	75%	2 days	6 Tb	350 bp	20 billion	1 to 500 ng	384	Current
	Helicos Bioscience Heliscope (Helicos Biosciences)	Lower	8 days	35 Gb	100 bp	20 million	100 ng	25	Company bankrupt
Sequencing by ligation	Ion proton, Complete Genomics (Thermo Fisher Scientific)	85%	2 to 4 hr	15 Gb	200 bp	80 million	50 ng to 1 μg	384	Current
	SOLiD (Thermo Fisher Scientific)	>99%	7 to 14 days	120 Gb	100 bp	2,400	ng	96	Current
Semiconductor with sequencing	Ion Torrent (Thermo Fisher Scientific)	>99%	2 hr	10 Mb to 1 Gb	600 bp	500	ng	8	Current
Real-time sequencing	PacBio SMRT (Pacific Biosciences)	>99%	30 hr	47 Gb	25 kbp	4 million	300 ng to 1 μg	96	Current
Nanopore	Flongle (Oxford Nanopore Technologies)	Lower	16 hr	1 to 2 Gb	4 Mb	100,000	10 pg to 1 μg	96	Current
	MinION (Oxford Nanopore Technologies)	Lower	72 hr	10 to 50 Gb	4 Mb	100,000	10 pg to 1 μg	96	Current
	GridION (Oxford Nanopore Technologies)	Lower	72 hr	10 to 50 Gb	4 Mb	100,000	10 pg to 1 μg	96	Current
	PromethION (Oxford Nanopore Technologies)	Lower	72 hr	100 to 300 Gb	4 Mb	100,000	10 pg to 1 μg	96	Current

*Q30 references the sequencing quality score. When the sequencing quality reaches Q30, virtually all of the reads will be perfect without errors or ambiguities. Q30 is considered a benchmark for quality in NGS.

Other potential benefits of molecular diagnostic strategies may yield more immediate rewards. Unlike traditional cultures, which take 3 to 10 days, delaying appropriate treatment, some molecular-based strategies can be rapid, particularly if guided by information around the clinically relevant pathogens of interest. In addition to improving the time gap from debridement to appropriate antibiotic selection (potentially preventing new biofilm formation), rapid pathogen identification could facilitate targeted intraoperative treatment approaches. Furthermore, the increased sensitivity and broad nature of DNA or RNA isolation (compared with the nutritional and environmental biases associated with culture) may identify additional pathogens that are clinically relevant, either on their own or when present in combination with others. However, there remain substantial gaps that must be addressed prior to translation into the clinical space.

Caveats to NA-Based Techniques

There are important caveats to keep in mind when evaluating the use of NA-based strategies, and specific details are needed so that study methodology can be critically evaluated. Table V summarizes critical methodologic data that are needed.

Dead Cells and/or Cell-Free NAs

Bacterial DNA may be present without viable cells (extracellular DNA). Viability can be confirmed by sending information to the microbiology laboratory for growth on specialized media.

Identification of Clinically Important Pathogen Features

Common microbiota from healthy human skin sites include some genera and species identical to known pathogens. Often, the 16S rRNA gene amplicon is not sufficient to differentiate between less problematic and more pathogenic strains (such

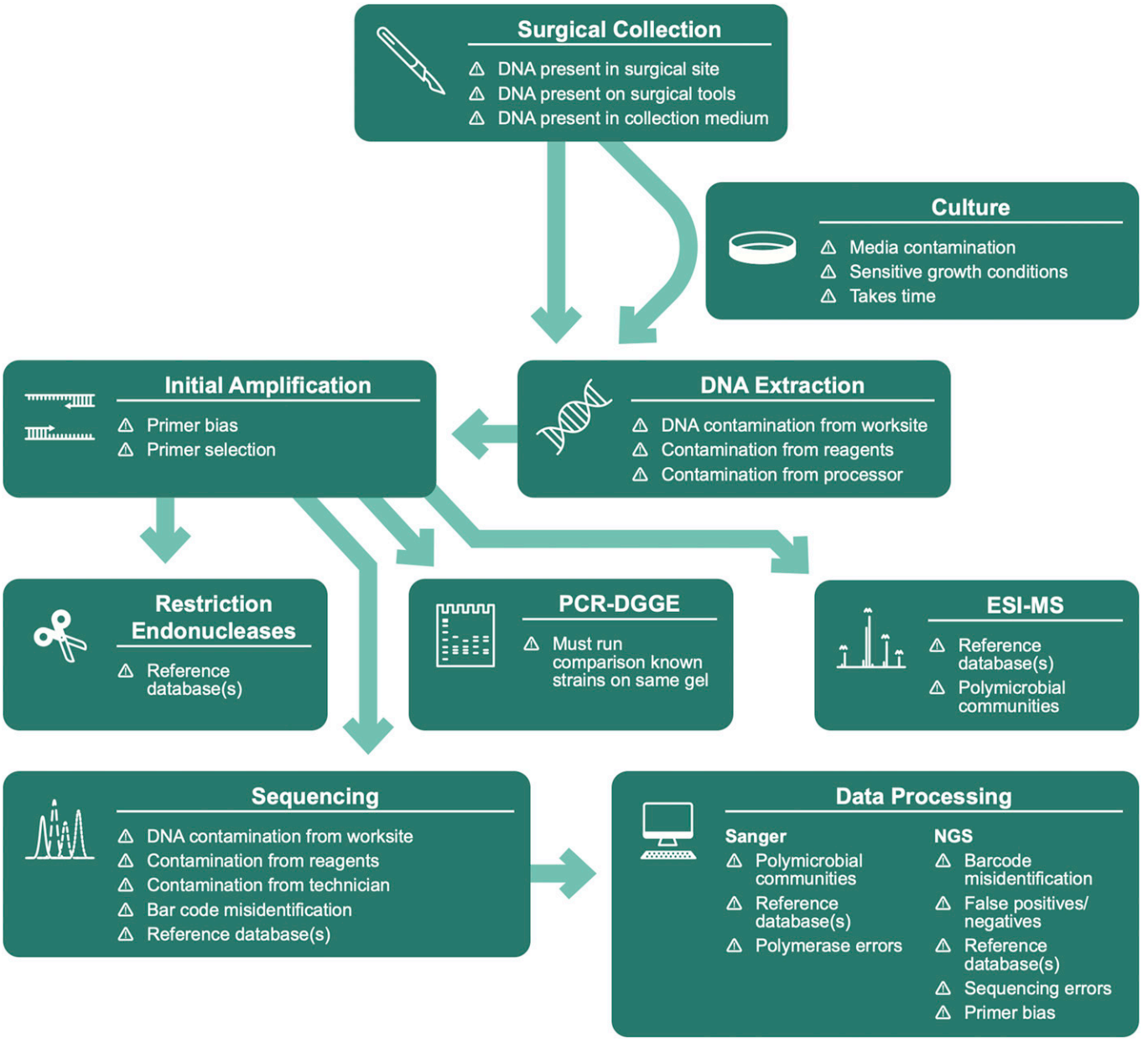

Fig. 2

Common sources of contamination and limitations or pitfalls that must be taken into account when using NA-based molecular techniques. DGGE = denaturing gradient gel electrophoresis, and ESI-MS = electrospray ionization mass spectrometry.

as methicillin-sensitive *Staphylococcus aureus* compared with methicillin-resistant *Staphylococcus aureus*). To handle this, many researchers use more than 1 NA-based technique to confirm the species or strain identity as pathogenic.

Mitochondrial Ribosomes

Mitochondrial ribosomes have sufficient similarity to bacterial ribosomes that primers designed to target bacteria will occasionally also amplify mitochondrial DNA. This can be particularly problematic in human samples, where human DNA vastly outnumbers microbial DNA.

Issues with Primers

Although 16S rRNA gene primers have been developed since the 1990s, no primer set is perfect. They are each known to have biases in which some taxa are more readily amplified than others. Potential primer biases must be considered when comparing data between experiments using different primer sets. Today, 16S rRNA gene-targeting primers designed for use with NGS applications have been tested to work well with most known clinical isolates. If using sequencing data to identify a novel pathogen, however, it is possible that primers may not be as efficient in amplifying its taxon[51]. When taxonomic identification

TABLE IV Information Necessary to Evaluate and Interpret Microbial Data Analysis*

Include with Analysis	Example(s)	Benefit	Problems if Absent
Extraction method	Kit-based In-house Automated	Easy comparison when same extraction methods are used	Extraction methods are optimized for different microbes. Harsher lysis techniques that may be necessary for spore-forming bacteria or fungi may be too harsh and degrade NAs from other microbes.
Positive extraction control	Standard microbial community	Confirms successful NA extraction	Low NA concentration may indicate failed extraction but be interpreted as low or no NA present.
Negative extraction control	Molecular biology grade water	Identifies contamination during extraction	High NA concentration may indicate contamination but be interpreted as sample with high NA abundance.
Positive PCR control	Standard DNA community	Confirms successful PCR amplification	No amplification may indicate failed reaction but be interpreted as no target present.
Negative PCR control	Molecular biology grade water	Identifies contamination of PCR reaction	Positive PCR reactions may indicate contamination but be interpreted as target present.
PCR reaction conditions	Salt concentrations Primer concentrations Enzyme brand name Thermocycling conditions	Reproducible amplification	Primer binding and enzyme efficacy can be susceptible to slight changes in reaction conditions. Future studies may fail if exact reaction conditions are not duplicated.
Primer names and sequences	Exact nucleotide sequences listed	Allows others to reproduce results in future samples	Results from studies targeting the same gene but with different primers may yield different conclusions based on primer specificity rather than biological differences.
Sequencing technology	Company and hardware and software version(s)	Different sequencing technologies have advantages and disadvantages (Table II), and results do slightly vary between technologies	Results from discontinued technologies may not be comparable with those from modern technologies.
Methods for reducing contamination	DNA extraction and post-PCR processing occurring in isolated areas	Assures reader that efforts have been made to minimize contamination	Reader may question if contamination occurred between samples.
Code for processing	GitHub repository	Reproducible analysis	Variation between data analysis may mask true variation in biological data or may falsely infer variations.
Deidentified raw data	.fasta files	Comparison with results from future studies	Nonreproducible results. Future studies must reproduce all sample types for direct comparison.
Define cutoffs or limit of detection thresholds	Minimum no. of reads to determine presence in a sample	Defines rare biosphere and the stringency of the study to account for false-positives or negatives	Low-abundance targets may be identified in some studies with low limits of detection while those with higher thresholds will miss them.
No. of reads (NGS)	Median reads per sample Variation in reads per sample	Too few reads may lead to false conclusion of microbe absence	Readers unable to determine depth of sequencing and validity of comparing rare biosphere between studies.
Normalization method for no. of reads (NGS)	Log_2 transformation Rarefaction	Standardize no. of reads per sample	Normalization methods may skew results; these skews may not be identified until future methods develop. Acknowledging the normalization method used will help future researchers to understand if they need to reprocess the data with new normalization techniques.
Define contaminants	Cutoff limits Patterns of abundance	Reproducible results	Contaminants may be identified as diagnostically important.

*When reading scientific literature, it is important to note if these items have been included. If information is not included in the research, the reader must acknowledge the problems that this absence may indicate. Sometimes the problem is merely an inability to compare with other literature, but other times, it may mean that the reported results should not be trusted until reproduced by other researchers.

TABLE V Grades of Recommendation*

Statement	Grade of Recommendation†
Positive and negative controls must be included	A
No. of reads per sample must be reported and any normalization method(s) described	B
Code used for data analysis should be publicly available	B
When comparing studies, the primers or targeted regions should be the same	B
Sequencing-based technology should be consistent when comparing studies	B
Ensure that the sequencing-based technology is currently maintained	I
Cutoff or limit of detection thresholds must be stated	B
Black-box methods should not be used	C
Publicly available, curated reference database(s) should be consulted	B

*Recommendations are based on the best evidence to date. †According to Wright[60], grade A indicates good evidence (Level-I studies with consistent findings) for or against recommending intervention; grade B, fair evidence (Level-II or III studies with consistent findings) for or against recommending intervention; grade C, poor-quality evidence (Level-IV or V studies with consistent findings) for or against recommending intervention; and grade I, insufficient or conflicting evidence not allowing a recommendation for or against intervention.

of uncultured bacteria to the species level is necessary, the 16S rRNA gene is sometimes insufficient. For example, Escherichia and Shigella cannot be differentiated by their 16S rRNA gene sequences alone. Taxonomic identification of bacteria via the 16S rRNA gene is dependent on comparing amplified sequences with a database of known sequences. Many public databases exist, each with different strengths and weaknesses in accuracy, coverage, taxonomic depth, and nomenclature.

Targeting Resistance and/or Virulence Genes

There are specific challenges associated with identifying resistance and/or virulence genes. Horizontal gene transfer spreads genes between phylogenetically distant bacteria. It is possible that simple amplification will detect genes of interest that are present in a specimen but not in the pathogenically relevant species. Naturally occurring mutations within the targeted primer-binding sites may also yield false-negatives. Additionally, for almost every mode of antibiotic resistance, there exist multiple responsible genes. It is not possible to design primers that will universally detect all resistance and/or virulence genes or even that will detect the same gene in all taxa. In cases where identification of a broad range of antibiotic resistance genes is necessary, metagenomic analyses are recommended over single-gene-targeted PCR.

Analysis of Complex Data

A new complexity for clinicians to consider is the large amount of data yielded from a single sample. These data may include community surveys of variation in a single gene (i.e., the

TABLE VI Specimen Storage Solutions and Their Suitability for Downstream Applications*

Storage Solution	Culture	Culture After Freezing	DNA	RNA
None[61]	+	—	+	—
Saline solution	++	—	+	—
Nutrient broth	++	—	—	—
Amies transport medium[62]	+++	+	++	—
15% glycerol[63-65]	+++	+++	++	—
Lysis buffer (i.e., Longmire)[63,64]	—	—	++	+
NA-stabilization solution (i.e., RNAlater[66])	—	—	+	+++
Phenol (i.e., TRIzol)[67]	—	—	++	+++
95% ethanol[68]	—	—	+	—
Formaldehyde or formalin[69,70]	—	—	+	—

*— = not recommended, + = possible but not ideal, ++ = good, and +++ = recommended for best results.

TABLE VII Clinical Utility of Each NA-Based Analysis Technique

Technique	Clinical Utility
End-point PCR	Good basic technique that will likely maintain utility
	Most useful for identification of specific targeted taxa and genes
	Adaptable for rapid point-of-care testing in the operating room
qPCR	Widely used in other clinical settings (e.g., SARS-CoV-2 testing)
	Useful to detect taxa or genes without first isolating bacterial cultures
	Adaptable for rapid point-of-care testing in the operating room
Sanger sequencing	Excellent technique for classifying or categorizing cultured microbes that cannot be identified using culture-based patterns
	Likely minimal clinical utility
	Rapid but dependent on first isolating pure culture
RNA sequencing	Currently used only in research; however, future clinical application may target identification of transcriptionally active bacteria
Amplicon-targeted NGS	Currently used primarily for research
	Can be considered for recalcitrant infection or when cultures are presumed to be inadequate (such as culture-negative infection); in this setting, results must be interpreted with extreme caution
	Potential for future clinical use as part of standard of care once issues have been addressed; some issues that must be addressed include, but are not limited to:
	• Shortening data generation and analysis time
	• Establishing "read" thresholds separating positive results from potential contaminants
	• Identifying pathogenic compared with non-pathogenic species
Metagenomic NGS	For research applications currently, but variations may be clinically relevant in future
	Future utility likely in identification of virulence or resistance genes present in infecting microorganisms; issues similar to those of amplicon NGS must be addressed prior to advancing into clinical practice

bacterial 16S rDNA gene) or broad community analysis of randomly amplified regions (i.e., metagenomic sequencing). The large amount of data produced by NGS necessitates more complicated data processing post-sequencing. This processing includes binding small sequences together (forming paired-end reads, scaffolds, and contigs) as well as comparing output sequences with existing databases (taxonomy assignment, scaffold testing, gene annotation)[52]. Based on this complexity, clinicians should consider caution when considering whether to use companies that market an ability to convert raw data to diagnostic results without offering insights into methods and protocols (black-box methods).

NGS

NGS is a powerful tool with incredible sensitivity that can hypothetically detect a single copy of a gene in 10 μL of a sample. Because such a small starting mass may yield a positive result, false-positives are a known confounding factor, particularly in samples with a low input mass (Fig. 2). There are several methods that can minimize this risk.

Each NGS technology has benefits and problems (Table IV). No single technology can concomitantly provide long amplicons, accurate reads, large numbers of reads, fast run time, and low cost using small sample inputs. Researchers must choose which of these components are most important to their application and must also consider whether the extra information received from NGS technology is worth the extra time, cost, and potential for a confounding diagnosis from detected, but not necessarily clinically relevant, pathogens.

Diagnosis based solely on NGS results is not currently recommended because of the risk of overdiagnosis (identifying the presence of bacterial taxa without confirming viability and/or pathogenicity) and subsequent overtreatment. Not enough studies have been performed to understand whether NGS can be used as a stand-alone diagnostic tool and how results should be interpreted. With that caveat acknowledged, when dealing with infections that have failed to respond to standard treatments, NGS may help to elucidate the presence of uncommon or previously undetected pathogens.

Best Practices for Collecting Specimens

Specimen collection and storage can affect NA-based diagnostic protocols. Solutions used in surgical treatment, especially antiseptics and disinfectants, may degrade NAs or inhibit enzymes. For this reason, specimens should be collected prior to any treatment. If collecting samples from multiple sites, it is important to ensure that no cross-contamination occurs. Ideally, specimens will pass directly into the collection medium. If intermediate surfaces are unavoidable, NA-free, or PCR-clean, supplies should be used. Standard materials may be rendered

TABLE VIII Suggested Negative Controls, Positive Controls, and Contaminant-Source Identification When Preparing Samples for NGS

	Negative	Positive	Contamination Source(s)
Collection	Sterile storage solution	Not commonly performed	Patient skin flora
			Irrigation fluid
			Instruments
			Clinician
Extraction	Reagents	Microbial community standard	Technician
			Reagents
			Environment
			Parallel samples
PCR	Water-only	Microbial community NA standard	Technician
			Reagents
			Environment
			Parallel samples
Sequencing	No PCR water	Successful PCR amplification	Technician
			Reagents
			Parallel samples
Processing	Empty primer indices	Previously processed data	Improper analysis
			Comparison database

PCR-clean via either treatment with RNase AWAY (Thermo Fisher Scientific) or bleach followed by rinsing with molecular biology-grade water[53] or treatment with autoclaving on an extended steam cycle of ≥80 minutes[54]. As discussed in the caveats section, even sterile items such as surgical drapes or gloves may harbor trace amounts of NAs[55] that will not harm patients but may contaminate specimens. The specific application used for analysis will influence the best collection medium and storage conditions (Table VI). If >1 type of test is to be performed on a specimen, it is usually better to take multiple samples from the same site and treat each independently.

Best Practices for Clinical Use

Although there is real potential for culture-independent diagnostic strategies to improve our diagnostic capacity by improving sensitivity and identifying microbial species that may be missed using traditional culture, these strategies are not yet ready for regular use as part of standard-of-care practice for several reasons. First, more carefully controlled microbiome-focused orthopaedic wound research must be performed to address outstanding questions with regard to the relationship between sensitivity and reproducibility in the detection of wound-associated microbes. This may necessitate closer collaborative relationships between orthopaedic surgeons and microbiome researchers, in lieu of commercial black-box microbiome sequencing providers. Second, there are important technical and logistical hurdles involving the infrastructure needed for the analysis of NA-based diagnostic tools, particularly NGS. Furthermore, the time required from the operating room to the data analysis and robust interpretation needed to inform clinical decision-making is currently impractically long. To date, most orthopaedic research applying NA-based techniques has been in arthroplasty, with a limited number of studies in trauma and orthopaedics more generally, and NGS is becoming increasingly important in these studies (see Appendix Supplemental Table 1). Newer advances in sequencing technology, particularly from Oxford Nanopore Technologies, and the rapid increase and spread of bioinformatics training among the biomedical workforce make the clinical use of NGS techniques in orthopaedic settings an optimistic goal for the coming years. Table VII outlines the clinical utility anticipated for these NA-based techniques.

Best Practices for Interpretation of These Complex Data Sets

There are several important metrics to keep in mind when evaluating research using NA-based technology. There are clear tradeoffs among speed, accuracy, sensitivity, price per sample, and coverage. Investigators and clinicians must carefully consider tradeoffs when selecting sequencing methods.

When evaluating published research and comparing results, it is important to keep the following in mind (Tables V and VIII):

1. Were appropriate positive and negative controls included at each step, and are these results reported? NGS studies should include positive controls sequencing communities of known composition and negative controls that sequence samples where no community is expected (Table VIII).
2. Is the number of reads per sample reported?

3. Is the code used for data analysis publicly available for other researchers to examine?
4. Are primers and/or targeted regions the same between compared studies? Both the efficacy and sensitivity of primers can be different during amplification and when comparing sequences with existing databases[56,57]. The ease of amplification of microbial groups changes with changes in primers, salt concentrations, temperatures, and other variables.
5. Is the sequencing technology consistent between studies? If not, how do biases of different technologies affect the results?
6. Is the technology currently maintained? Technologies present in published literature for only a short period of time must be treated with skepticism.
7. The cutoff or limit of detection thresholds should be stated along with definitions of contaminants. Whenever possible, deidentified raw data should be publicly available so that another researcher may repeat the analysis or compare the raw reads with those from samples produced in future studies.
8. Achieving NGS data with the exact same number of reads per sample is impossible and the read counts can vary quite a bit between specimens[58]. In experiments containing an uneven number of reads per sample (a >10-fold difference), the researcher must consider resequencing outlier samples or normalize the data to compare samples more accurately using strategies such as rarefication.
9. Methods sections of published papers should include description of methods applied to reduce false-positives, such as experimental controls to reduce the identification of false-positives, well-defined threshold of reads per sample (≥2,000)[59], removal of taxa present in samples in only 1 or 2 reads, and removal of taxa whose abundance is linearly related to the volume of the samples analyzed.

Conclusions

Molecular diagnostic strategies will become increasingly important in the diagnosis of infection and identification of pathogens, both in research and in clinical practice. However, for these techniques to be effectively applied to orthopaedics, clinicians and clinician-scientists must better understand the nuances, appropriate applications, and the limitations associated with each of these assessment tools. We anticipate that this review may provide a mechanism for generating hypotheses, improving standards, designing better studies, and enhancing our ability to effectively interpret and apply published research.

Appendix

eA Supporting material provided by the authors is posted with the online version of this article as a data supplement at jbjs.org (http://links.lww.com/JBJS/H421). ■

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