The combination of two or more markers to detect PJI has been studied. It has been shown that the combination of synovial fluid α-defensin and CRP provided a sensitivity of 97% and a specificity of 100% in diagnosing PJI [17]. The combined use of synovial CRP and adenosine deaminase (ADA) improves the positive predictive value [18]. A synovial fluid CRP should be included in the synovial fluid analysis and correlated with other lab markers [17].

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QUESTION 8: What is the role of molecular techniques for detection of pathogen deoxyribonucleic acid (DNA) (polymerase chain reaction (PCR) or next-generation sequencing) in patients with infected total ankle arthroplasty (TAA)?

RECOMMENDATION: Molecular techniques, particularly next-generation sequencing and the Ibis T5000 technology, have the potential to be used as an important adjunct in the diagnosis of bacterial infection following TAA, although sufficient clinical evidence is lacking.

LEVEL OF EVIDENCE: Limited

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

RATIONALE

The culture of multiple periprosthetic tissue samples is currently considered the gold standard for microbiological diagnosis of periprosthetic joint infections (PJIs) [1]. However, biofilm-associated infections are not easily detected by culture-based methods and are often resistant to conventional antimicrobial therapy. Therefore, it seems imperative to promptly investigate and subsequently integrate molecular diagnostic techniques into the clinical practice for the management of PJI [2].

The most common molecular techniques that have been used to diagnose PJI are both based on PCR: specific PCR and broad-range PCR [3]. Specific PCR targets a single bacterial species (e.g., *Staphylococcus aureus*) or a group of closely-related species (e.g., all staphylococcal species). These are typically considered real-time PCR assays. Specific PCRs can be used in the diagnosis of any targeted pathogen with extreme sensitivity, potentially detecting even a single copy of the target DNA. This approach provides accurate results within hours and has the advantage of singling out any organisms deemed as significant, thereby making contamination easier to control for, as well as making quantification possible [3].

Broad-range PCR, in contrast to specific PCR assays, provides the opportunity to detect DNA from any pathogen rather than a specific preset of expected pathogens. Almost all broad-range PCR techniques utilized in diagnostic microbiology laboratories are based on the gene coding for the small subunit of the bacterial ribosome (16S rDNA). The main limitations of broad-range PCR relate to inherent problems with contamination and sensitivity. Contamination arises from bacterial DNA present in PCR reagents or inadvertently introduced during the collection and handling of the sample, particularly if additional fluids are added to the culture sample during transport or laboratory processing [4]. Unfortunately, these "contaminant" bacteria detected with broad-range PCR are closely related to the microorganisms that cause low-grade

PJI, making the distinction between true-positive versus false-positive PCR results challenging. For these reasons, broad-range PCR has not yet been integrated into the standard routine diagnostic procedure of PJI by most laboratories, but this technique is a valid option to be applied to the diagnosis of synovial fluid or periprosthetic tissue infections [5,6].

Comparing the specific and broad techniques, one study found the sensitivities of specific PCR for detection of *Propionibacterium acnes* and *staphylococcus spp*. in sonication fluid from prosthetic shoulder infections to be 89% and 97%, respectively [7]. In contrast, broad-range PCR of tissue cultures in patients with PJI has previously demonstrated a sensitivity of only 50% [8].

The arrival of high-throughput (next-generation) sequencing techniques has enabled the generation of thousands of individual sequences from a single broad-range PCR [3]. This approach seems to be promising in aiding in surgical site infection and PJI detection, since it provides detailed information on the bacterial population present in prosthetic joint samples [3]. The next-generation technique of pyrosequencing allows for massively parallel, rapid identification of pathogens at a much lower cost per base than the traditional sequencing. The greater breadth and depth of pyrosequencing, in which hundreds of thousands of sequences can be generated in a single run, means that low abundance species have a higher chance of being detected [3].

When comparing molecular and microbiological techniques on PJIs, culture and PCR have shown similar sensitivities (72.6% and 70.4%) and specificities (98.3% and 97.8%) [9,10]. While using a combination of 16S rDNA PCR and lateral flow immunoassay, the 16S recombinant DNA (rDNA) test system provided a diagnostic result within 25 minutes in 97% of all patients. This can be juxtaposed to the microbiological culture of synovial fluid, which achieved a lower sensitivity than that of the 16S rDNA test with 80% [11]. In terms of cost, molecular diagnosis may be a more expensive diagnostic method than bacterial culture with a cost-effectiveness that has not yet been evaluated [12]. The direct detection of bacterial 16S rDNA shows encouraging results and warrants further evaluation in larger patient cohorts [11].

While molecular techniques have shown to be important in diagnosing PJI in orthopaedic fields other than foot and ankle, they have not been well-studied in the setting of an infected TAA. In one of the few studies identified studying the utilization of molecular techniques in the foot and ankle, Stoodley et al. evaluated several techniques to ascertain the presence of a bacterial infection in an explanted TAA that had an initial negative culture. The techniques included the Ibis T5000, real time-polymerase chain reaction (RT-PCR), a direct culture of the ankle hardware, confocal microscopy, and fluorescent in situ hybridization (FISH) [13].

The Ibis T5000, a research use only (RUO) technology based on the combination of PCR amplification of highly conserved pathogen genomes with high-performance electrospray ionization mass spectrometry and base-composition analysis, is able to tease out a variety of organisms (including bacterial and viral) down to the species level [14]. Data points include number of genome copies, relative organism abundance and antibiotic sensitivity [15,16]. Based on Ibis testing, Stoodley et al. were able to identify the presence of *S. aureus*, *S. epidermidis* and the methicillin-resistant *mecA* gene in tissue on the removed TAA hardware [13]. Additionally, the Ibis detected that there was close to ten times more *S. aureus* in comparison to the *S. epidermidis*. Of all the techniques investigated, the authors proposed the Ibis T5000 technology to have the most potential in aiding with clinical detection of PJI with TAA [13].

In addition to the Ibis system, the authors used RT-PCR in order to detect metabolically active *S. aureus* [13]. The authors were able to harvest ribonucleic acid (RNA) from a tissue sample and after converting the RNA to complementary DNA via reverse transcription, they utilized specific PCR primers for the bacterial glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and histidine ammonia-lyase (*hutH*) genes [17–19]. The study demonstrated the presence of *S. aureus* messenger RNA for both the GAPDH and the *hutH* genes [13].

Another technique was a direct culture of the tibial metal component of the removed ankle hardware. After a detailed agar preparation protocol, the tibial component was placed in a beaker in which an agar formed. After incubation, the number of bacterial colony-forming units (CFUs) on the agar was eventually estimated. The authors reported approximately 1000 CFUs spread across the entire tibial component and composed of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *S. epidermis* [13].

Confocal microscopy was also implemented for viability determination after staining and using a 488nm excitation wavelength to identify bacteria as living or dead. Fluorescent in situ hybridization (FISH) was also utilized using fluorophore-labeled 16S rDNA sequences specific for *S. aureus* [20–22]. A red Syto59 fluorescent nucleic acid stain was used to stain all bacterial and host nuclei, allowing *S. aureus* to be the only species stained both red and green. Bacteria that were stained with Syto59 solely were distinguished from host nuclei on the basis of size [22,23]. Confocal microscopy and FISH demonstrated a scattered distribution of biofilm formation, with clusters of bacterial colonies on tissue, the talar component edges, the polyethylene bearing surface and the tibial component. FISH testing also indicated that bacterial growth was predominantly *S. aureus* and *S. epidermidis* to a lesser extent [13].

These findings presented by Stoodley et al. offer to be an important diagnostic step to gauge the presence of a bacterial infected TAA [13]. However, further research is necessary to decide the true clinical utility of these techniques.

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QUESTION 9: Should culture samples be taken during all revision total ankle arthroplasty (TAA)?

RECOMMENDATION: We recommend that intraoperative cultures be taken during revision TAA. The result of intraoperative cultures should be interpreted together with clinical suspicion for infection and the results of the laboratory and imaging investigations. We also recommend that multiple tissue specimens be collected. Given a lack of evidence for routine intraoperative cultures for revision TAA literature, this recommendation is based on analogous evidence in the total hip and knee replacement literature.

LEVEL OF EVIDENCE: Consensus

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

RATIONALE

There have been no studies in the TAA literature that have evaluated the utility of routine intraoperative cultures for all revision TAA cases. Multiple case series and review articles on revision TAA have been published which do not specifically advocate for or against this practice [1–4]. Jonck et al. do, however, recommend curettage of any encountered cysts at the time of revision and advise that cyst material should be sent for cell count, microbial culture and histopathology [3]. However, no data is included regarding previous results and utility of these samples.

There have been multiple studies in the total hip and knee replacement literature investigating the role of routine cultures taken during revision arthroplasty for presumed aseptic failure. Barrack et al. published on a series of revision total knee replacements with unexpected positive intraoperative cultures [5]. There were 41 cases with positive cultures out of 692 total cases. Twenty-nine of these cases had only one positive culture without additional evidence of infection and were considered false positives. None of the presumed false positives had long-term signs of infection or required additional surgery. The other 12 cases had multiple positive cultures or one positive culture and an abnormal preoperative inflammatory marker or synovial aspirate. These cases were treated with a four to six week course of antibiotics and two of these patients presented with early recurrent infection requiring a two-stage exchange. An additional patient had aseptic loosening requiring revision at six years, at which time there was no sign of infection and negative intraoperative cultures. The authors recommended routinely sending at least five sets of cultures in the setting of abnormal preoperative inflammatory markers, abnormal synovial aspirate or tissue appearing concerning for infection intraoperatively at the time of revision.

Jacobs et al. reported on 679 cases of revision hip or knee arthroplasty for presumed aseptic failure [6]. Infection was defined by the presence of two or more positive intraoperative cultures with the same organism. The incidence of unsuspected infection was 10%. For total knee replacements, patients diagnosed with infection went on to require repeat revision for recurrent infection at a higher rate compared with patients who were not diagnosed with infection at initial revision. For total hip replacements, there was no significant increased rate of recurrent infection requiring revision. The authors emphasized the importance of improved preoperative work-up prior to revision total joint arthroplasty to minimize the number of unsuspected prosthetic joint infections.

Given that there is a small but significant incidence of unsuspected joint infection in hip and knee arthroplasty, there is likely a similar incidence of unsuspected TAA infection amongst presumed aseptic failures. Routine cultures at the time of revision for aseptic failure may help to identify unsuspected infections. However, even the literature for hip and knee replacement does not provide significant evidence to suggest how to intervene once the diagnosis is made and whether long-term outcomes can be improved once intraoperative cultures lead to the diagnosis of periprosthetic joint infection (PJI).

Therefore, we recommend that all patients undergoing revision ankle arthroplasty be investigated for PJI, which includes measuring serum markers, aspiration of the joint, intraoperative evaluation (which may include histology) and any other necessary tests. The