HK38: Is there a role for antibody testing for microorganisms in the diagnosis of periprosthetic Joint Infection?

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Response/Recommendation:

Antibody testing for microorganisms is a potential adjunct for the diagnosis of a periprosthetic joint infection (PJI). More studies are needed to determine the most appropriate microbial targets for antibody testing and to evaluate the clinical performance of this approach.

Level of Evidence: Moderate

Delegate Vote:

Rationale:

Accurate and early diagnosis of periprosthetic joint infection (PJI) is of paramount importance in establishing a management plan for antimicrobial therapy and revision surgery. However, the diagnosis of PJI remains a major clinical challenge, primarily due to the limitations of traditional diagnostic tools, such as microbiological cultures, cell numbers in joint aspirate,

imaging, histology, and inflammatory markers (erythrocyte sedimentation rate, ESR; C-reactive protein, CRP), which can yield inconclusive results, especially in chronic, low-grade, or polymicrobial infections, and are subject to limitations due to specimen collection, low bacterial burden, and prior antibiotic treatment.

Antibody testing for microorganisms in PJI has been explored in recent years for its ability to detect pathogen-specific antibodies, potentially providing a non-invasive diagnostic option. Such a serodiagnostic test should ideally have high specificity to distinguish PJI from aseptic conditions, such as mechanical loosening or aseptic inflammation, and to differentiate between microbial contamination and infection. In addition, it should have a high sensitivity to be able to detect low-grade infections and culture-negative cases. Ideally, it would allow us to monitor secondary bacterial invasion during treatment, indicate the optimal timing of re-implantation, and ultimately guide targeted antimicrobial therapy.

This review explores the role of antibody testing for microorganisms and its efficacy in the diagnosis of PJI, based on findings from 18 key studies investigating its sensitivity, specificity, clinical relevance, and diagnostic potential. Initially, studies evaluated the potential of serological assays using single bacterial antigens for the diagnosis of PJI. Examples were exocellular carbohydrate antigens [1] and poly-N-acetyl-ß-(1,6)-glucosamine (PNAG, [2]) from *Staphylococcus (S.) epidermidis*, staphylococcal slime polysaccharide antigens (SSPA, [3]) and exocellular glycolipid antigen [4] from *S. aureus*, and streptolysin O from *Streptococcus pyogenes* [5]. Using conventional ELISA tests, these studies demonstrated the value of this approach for the diagnosis of PJI and other orthopaedic infections caused by staphylococci and streptococci, respectively.

Including complex protein mixtures led to increased sensitivity of the assay. Using the ELISA method, Wang et al. found that sera from infected guinea pigs and patients who have implant-associated infections had higher levels of IgG antibodies to extracellular proteins from staphylococcal biofilms than controls [6]. Another study investigated antibody production against several *S. aureus* biofilm-upregulated antigens (SAOCOL0486, glucosaminidase, and SACOL0688) for the detection of chronic *S. aureus* infection using both ELISA and lateral flow assay (LFA) in sera from rabbits with *S.* aureus-mediated osteomyelitis and in synovial fluid from humans who have PJI [7]. The authors demonstrated the clinical diagnostic utility of the SACOL0688 antigen for the diagnosis of *S. aureus* infection with high specificity (100%) and sensitivity (100, 91%) using LFA and ELISA, respectively [7].

Recent advances in the field of infection immunology led to the replacement of conventional ELISA with bead-based multiplex immunoassays based on the Luminex® technology, providing a high-throughput platform for studying infection processes. In their 2015 study, Nishitani et al. characterized the antibody response in patients who have *S. aureus* culture-confirmed deep musculoskeletal infections against 14 selected *S. aureus* antigens (Gmd, Amd, SrtA, IsdA, IsdB, IsdH, ClfA, ClfB, FnbpA, coagulase, Hla, SCIN, CHIPS, and Efb) [8]. Using multivariable logistic regression analysis, they combined the IgG titers against all 14 antigens and determined a sensitivity of 80.0% and specificity of 92.5% for detecting patients who have infection.

Using a similar panel of eight staphylococcal antigens (IsdA, IsdB, IsdH, SCIN, CHIPS, Hla, and the Atl subunits Amd and Gmd), Muthukrishnan *et al.* found that higher serum antibody levels specific for Amd, Gmd, Hla, SCIN, CHIPS, and IsdH at the time of diagnosis could predict a better outcome of the orthopaedic infection [9]. Interestingly, in another study looking at antibody levels to the same antigens up to 12 weeks after primary total knee arthroplasty, the

authors observed a reduction in anti-staphylococcal antibodies on postoperative day one, which they attributed to blood loss from surgery and hemodilution with intravenous fluid. According to the authors, this observed reduction in humoral immunity may explain early postoperative infections [10].

In a further study, the authors utilized a bioinformatic approach, employing antibodies derived from newly activated, pathogen-specific plasmablasts in human blood (*medium enriched for newly synthesized antibodies*, MENSA) to identify six functionally distinct *S. aureus* antigens (IsdB, IsdH, Gmd, Amd, SCIN, and Hla) that can be used as diagnostic biomarkers for *S. aureus* musculoskeletal infections, exhibiting high potential for discriminating between culture-confirmed *S. aureus*-infected patients and healthy controls [11]. Furthermore, the MENSA approach has been shown to have high diagnostic potential for monomicrobial *S. aureus* and *Streptococcus agalactiae* orthopaedic infections [12].

In 2016, Marmor *et al.* designed a panel including 16 recombinant antigens from different microorganisms commonly associated with PJI, including *S. aureus*, *S. epidermidis*, *S. lugdunensis*, *Streptococcus agalactiae*, and *Cutibacterium acnes* [13]. Utilizing a comparative immunoproteomic approach, the immunoassay demonstrated satisfactory performance, exhibiting a sensitivity of 72.3% and a specificity of 80.7% for detecting staphylococcal infections. Interestingly, the sensitivity increased for staphylococcal infections that occurred more than three months following arthroplasty and among patients exhibiting elevated ESR or CRP levels [13, 14].

Since this multiplex serological test, which had the capacity to detect antibodies against multiple pathogens simultaneously, proved useful in the diagnosis of PJI, it has been commercialized with the aim of providing rapid test results (approximately two hours). This immunoassay has been tested in two different prospective studies [14, 15], with the objective of assessing its contribution to the routine diagnosis of chronic PJI. Despite its favorable performance, with sensitivity and specificity rates of 86.7 and 96.2%, respectively [15], the test was unable to definitely confirm the diagnosis of PJI in a multicenter prospective observational study involving 115 orthopaedic patients [14]. However, the approach was deemed useful for the post-therapeutic follow-up of patients who have staphylococcal PJI in a prospective clinical study, where a decrease in the level of antibodies was indicative of successful treatment [16]. Moreover, combining the measurement of serum antistaphylococcal antibodies (SASA) with synovial fluid culture results improved the ability of preoperative aspiration to diagnose staphylococcal PJI of the replaced hip [17].

The use of species-specific multiplex immunoassays for the identification of causal pathogens in PJI has gained much interest, and Janz et al. have successfully implemented this approach for simultaneous measurement of antibody specificities against 32 pathogens commonly associated with PJI in a prospective pilot study [18]. Utilizing their multiplex approach, they were able to trace the dynamics of the pathogen-specific humoral immune response and employed the decline in pathogen-specific IgG binding as an indication of successful treatment, thus introducing a promising tool for PJI monitoring [18].

Whilst multiplex antibody testing shows promise, it is not without limitations. One challenge is the variability in test performance depending on several factors, such as the infection stage (acute vs. chronic infection) [7, 13–15], the type of assay used (LFA, ELISA, and multiplex assay) [7, 8], the targeted pathogen [12–15], choice of target antigens (e.g., biofilm antigens) [7], sample material (serum, synovial fluid, or MENSA) [7, 11, 12], the inclusion and exclusion criteria of the study subjects (some excluding culture-negative infections), and the immune

status of the patient. For instance, patients who have immune deficiencies or those who are on immunosuppressive therapy may have a reduced antibody response, leading to false negatives [11]. Furthermore, it is important to note that antibody testing measures a systemic immune response to a pathogen, but does not indicate the site of infection. This limitation might be addressed through the use of joint aspirates to test for pathogen-specific antibodies [7].

Despite the encouraging results obtained thus far, there is an imperative for further enhancements in the sensitivity of current multiplex assays, particularly in the context of acute infections and those caused by less prevalent organisms. Moreover, the execution of large-scale clinical trials is crucial to substantiate the feasibility of fully incorporating these tests into clinical practice for the diagnosis of PJI across a range of patient populations, including those with comorbidities or prior antibiotic or drug treatment, which may influence antibody production. Furthermore, there is a need to establish the cut-off values required to define serology as positive, as these vary between studies [3–6, 18]. In addition, studies examining the impact of antibody testing on patient outcomes, including recovery time, antibiotic stewardship, and surgical decision-making, are essential to fully understand the clinical benefits of these tests. A cost-benefit analysis is also required in order to evaluate the economic usefulness of antibody testing. A combined approach, integrating antibody testing with other diagnostic tools such as PCR, next-generation sequencing, and advanced imaging techniques, may improve diagnostic accuracy and help tailor more effective treatment strategies for PJI.

Notwithstanding the aforementioned limitations, serum antibody testing for microorganisms still has a role in the diagnosis of PJI. However, it can be used as an adjunct rather than a standalone diagnostic method. It is a non-invasive and rapid tool that could be most effectively used as part of a comprehensive diagnostic strategy that includes microbiological cultures, inflammatory markers, clinical assessment, and imaging, thus enhancing the overall diagnostic accuracy and facilitating earlier detection of infection, particularly in challenging cases, where traditional diagnostic methods fall short, and also to monitor the efficacy of infection resolution [17, 18]. Numerous studies have demonstrated the efficacy of antibody testing in a variety of orthopaedic infection settings, including PJI, fracture-related infections, native joint septic arthritis, osteomyelitis, orthopaedic infections in the soft tissue, and diabetic foot infections [5, 7, 9, 11, 12]. Multiplex antibody testing could be designed to detect polymicrobial infections, a common challenge in diagnosing PJI. This objective can be realized by incorporating targets from various pathogens to encompass a more extensive range of organisms, as previously documented [13–15, 18]. For instance, Sulovari et al. demonstrated that MENSA possesses diagnostic potential for Group B Streptococcus polymicrobial musculoskeletal infections [12]. This is of particular importance in cases where microbiological cultures fail to identify all pathogens present. The ability to rapidly detect pathogen-specific antibodies could help clinicians make more informed decisions about treatment, including the selection of appropriate antibiotics and determining the need for surgical intervention [7, 18].

Conclusions:

The studies reviewed demonstrate that serological tests targeting pathogen-specific antibodies, while unable to localize an infection due to their systemic nature, offer several benefits, including their flexibility, since they can cover a wide spectrum of microbial strains that can be easily expanded according to the choice of targets, as well as their robustness against antimicrobial therapy. As technology and assay sensitivity improve, antibody testing may play an increasingly important role in the diagnosis and management of PJI.

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