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REVIEW ARTICLE

Advancements in Diagnosing Periprosthetic Joint Infections after Total Hip and Knee Arthroplasty

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Abstract: Periprosthetic joint infection (PJI) is a complication of total joint arthroplasty that is challenging to diagnose. Currently, there is no "gold standard" for definite diagnosis of PJI. A multi-criteria definition has been described for PJI based on microbiology cultures, serum markers, such as erythrocyte sedimentation rate and C-reactive protein (CRP), synovial fluid biomarkers, such as leukocyte esterase and histopathology assessment of the periprosthetic tissue. The conventional serum markers are generally nonspecific and can be elevated in inflammatory conditions. Therefore, they cannot be relied on for definite diagnosis of PJI. Hence, with the use of proteomics, synovial fluid biomarkers such as α-defensin, IL-6, and CRP have been proposed as more accurate biomarkers for PJI. Current methods to culture micro-organisms have several limitations, and can be false-negative and false-positive in a considerable number of cases. In an attempt to improve culture sensitivity, diagnostic methods to target biofilms have recently been studied. The understanding of the concept of biofilms has also allowed for the development of novel techniques for PJI diagnosis, such as visualizing biofilms with fluorescent in-situ hybridization and detection of bacteria via DNA microarray. Lastly, the use of amplification-based molecular techniques has provided methods to identify specific species of bacteria that cause culture-negative PJI. While diagnosing PJI is difficult, these advances could be valuable tools for clinicians.

Keywords: Advancements, Arthroplasty, Biofilms, Diagnosis, Molecular diagnostic techniques, Prosthesis-related infections, Serum markers, Synovial fluid markers.

INTRODUCTION

Due to the increase in the number of individuals undergoing joint replacement procedures, a concomitant rise in the number of complications is expected [1]. There are many different complications that can occur after total joint arthroplasty, the most devastating of which is periprosthetic joint infection (PJI), which may require multiple surgical procedures and long-term antibiotic therapy, and rehabilitation [2]. Therefore, PJI may have an immense impact on the health and function of patients and can impose a considerable financial burden on the healthcare [3]. Based on projection studies, it is anticipated that the number of patients presenting with PJI is on an exponential increase.

A wide array of bacterial genera and species can cause PJI. Gram-positive bacteria, particularly *Staphylococci* and *Streptococci*, are responsible for the majority of PJI cases. Other pathogens including Gram-negative bacteria, anaerobes, fungi, mycobacteria, and other bacteria such as propionibacteria and acinetobacter species have also been implicated in causing PJI [4].

Multiple diagnostic tests are currently available that may help in determining the cause of failure of a prosthetic joint. While the clinical diagnosis of PJI is not always straightforward, the lack of a gold standard test makes its diagnosis challenging [5]. Clinical history and examination do not always distinguish between septic or aspetic cause of failure. Thus, it is not uncommon to encounter cases of so called "aseptic failure" that were indeed infected which were either not investigated properly prior to revision or had escaped detection using the currently available methods for

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diagnosis of PJI.

Multi-criteria definitions have been created to rectify this problem. Table **1** Additionally, these criteria provide a consistent template for research purposes, such as making it easier to compare the efficacy of various tests and methods to diagnose PJI. In 2011, the Musculoskeletal Infection Society (MSIS) Workgroup published their definition for PJI [5], which was recently modified by the International Consensus Group (ICG) on PJI [6]. Another organization, namely the Infectious Disease Society of North America, has also proposed a definition for PJI that appears to differ from that of the MSIS and ICG in some aspects [7].

Table 1. Definitions of PJI*.

International Consensus Group (ICG) on PJI * (2)	Infectious Disease Society of America (IDSA) (4)
One of the following **major criteria** must be met for diagnosis of PJI: 1. Two positive periprosthetic cultures with phenotypically identical organisms, or 2. A sinus tract communicating with the joint, or **Three** of the following **five minor criteria** must be met for the diagnosis of PJI: 1. Elevated serum C-reactive protein (CRP) AND erythrocyte sedimentation rate (ESR) 2. Elevated synovial fluid white blood cell (WBC) count OR ++ change on leukocyte esterase test strip 3. Elevated synovial fluid polymorphonuclear neutrophil percentage (PMN%) 4. Positive histological analysis of periprosthetic tissue 5. A single positive culture	PJI is **definitely** present if: 1. There is a sinus tract that communicates with the prosthesis, or 2. There is purulence around prosthesis without any other known cause PJI has a **high chance** of being present if: 1. Cultures grow a virulent microorganism from tissue or synovial fluid samples 2. The pathologist see's acute inflammation when examining the debrided periprosthetic tissue. 3. There are two or more cultures with the same organism, including genus and species or common susceptibility to antibiotics. This can be two or more intraoperative cultures or a combination of intraoperative cultures and pre-operative synovial fluid.

* PJI may still be present if these criteria are not met, so clinicians are urged to use their best judgment in making the final diagnosis.

* This definition is a modification of definition proposed by the Musculoskeletal Infection Society (MSIS). The major difference is that the ICG did not consider purulence as a minor criterion and the leukocyte esterase strip test was added as an alternative for synovial fluid WBC count. Moreover, the diagnosis of PJI can be made with the presence of three out of five minor criteria, as above, instead of four out of six minor MSIS workgroup criteria.

Although these definitions share some of their criteria, they are considerably different in terms of the weight they assign to some criteria. While there is no universally accepted definition of PJI, the ICG definition of PJI is currently used by many clinicians, societies, and organizations worldwide, and has also been adapted by the Centers for Disease Control [6]. Nevertheless, PJI may still be present, even in the absence of sufficient criteria for infection, and a systematic diagnostic approach should therefore be combined with an individualized therapeutic strategy.

There have been considerable efforts recently to identify novel biomarkers and methods to more easily and effectively diagnose PJI. Some of these tests and techniques show promise for the accurate diagnosis of PJI and others allow for isolation of the causative microoragnisms. In this article, we will review the evolving and novel advancements in diagnosing PJI after total joint arthroplasty.

SERUM BIOMARKERS

Blood biomarkers are attractive alternative methods for the diagnosis of PJI mainly because of the ease of sample collection and avoidance of joint aspiration. Routine blood markers, namely erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are not sufficiently specific to diagnose PJI. ESR and CRP as a combination test is sensitive (96%) for detection of PJI yet its specificity is low (56%) [8], as both ESR and CRP can be elevated due to an underlying inflammatory condition such as autoimmune disorders, malignancies, concurrent infections, or in the early postoperative period. Considering the high sensitivity of ESR and CRP and their low cost, they are recommended as screening tests for PJI [8, 9]. Nevertheless, even normal levels of ESR and CRP do not rule out PJI, and these tests alone should not be relied on for definite exclusion of PJI [10].

Recently, numerous serum biomarkers have been studied for the diagnosis of PJI. These mainly include inflammatory biomarkers such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), soluble intercellular adhesion molecule-1 (sICAM-1), Ig-G antibodies to short chain exo-cellular lipoteicholic acid, and procalcitonin [11 - 15]. These inflammatory cytokines are secreted by various inflammatory cells (neutrophils, monocytes, macrophages, T2-lymphocytes, and fibroblasts) during response to infectious and non-infectious stimuli such as aseptic loosening (local release) and postoperative systemic inflammatory response [16 - 18]. IL-6 was initially presented as a highly sensitive and specific marker for PJI [19, 20]. However, there are concerns about the selection bias of these studies, as they did

not consider the confounding influence of previous antibiotic use and associated inflammatory conditions on IL-6 and other inflammatory markers [19]. Procalcitonin has also been used as a marker of systemic infection, but its role in the diagnosis of a local infection such as PJI is limited because the threshold of procalcitonin in patients with local infection overlaps significantly with its normal range (low specificity) [13, 14, 21]. Nevertheless, recent studies did not confirm that IL-6 and procalcitonin outperform conventional blood biomarkers for diagnosis of PJI [22].

SYNOVIAL FLUID BIOMARKERS

Synovial fluid biomarkers can be considerably helpful in the diagnosis of PJI and improve the accuracy of other tests such as serum biomarkers. Synovial fluid white blood cell (WBC) count and differential are currently minor criteria in the definition of PJI as proposed by the International Consensus Group. In recent years, however, numerous biomarkers have been investigated for patients with PJI, including inflammatory cytokines (such as interleukins 1, 6, 8,10, and 17, TNF-α, Interferon-γ, resistin, and thrombospondin), inflammatory reactive proteins (such as CRP), bactericidal leukocyte enzymes (such as esterase, elastase, and bactericidal/permeability-increasing protein, gelatinase-associated lipocalin, and lactoferrin, all of which are present in polymorphonuclear leukocytes), markers of angiogenesis (such as vascular endothelial growth factor) and antimicrobial proteins (such as α-defensin, β-defensin, and cathelicidin LL-37) [23 - 26]. Interestingly, many of these synovial fluid biomarkers did not have any correlation with synovial WBC count, so these synovial fluid markers are not simply surrogate markers for an increase in local inflammation in the joint as a result of a PJI. Additionally, it was found that the markers that had the highest specificity and sensitivity were proteins that have antimicrobial properties, which is likely the reason for their increased concentration in synovial fluid during PJI. Since the mechanism of action for these biomarkers is different than that of currently used tests, these biomarkers hold great promise for a novel approach in diagnosing PJI [27]. The main disadvantage of synovial biomarkers is that these tests depend on the availability of synovial fluid, and synovial fluid cannot be aspirated from a joint in all PJI cases. Moreover, some of the inflammatory biomarkers may represent any type of inflammatory process in the prosthetic joint such as an adverse reaction to foreign material. Therefore, these tests may not be specific enough for PJI.

- ***Synovial CRP*** Although serum CRP (secreted by the liver) is elevated as part of the systemic response to PJI, recent studies show that the synovial CRP is also increased in PJI patients and is actually more accurate than serum CRP [25, 32]. In a retrospective study of 66 patients undergoing revision total knee arthroplasty (TKA) the sensitivity of synovial and serum CRP was 84% and 76%, respectively, and their specificity was 97% and 93%, respectively [32]. A recent publication demonstrated that combined CRP and α-defensin in the synovial fluid with use of enzyme-linked immunosorbent assay provides sensitivity and specificity of 97% and 100%, respectively, based on the MSIS criteria as the standard definition for PJI. Moreover, the accuracy of the combined test remained at 99%, even with the inclusion of patients with systemic inflammatory diseases and those with previous antibiotic consumption [33].
- ***Leukocyte Esterase (LE)*** is an enzyme secreted by neutrophils that are recruited in the synovial fluid as a response to PJI [28, 29]. The test includes a rapid colorimetric strip and has been used to detect infection in other bodily fluids such as urine and pleural fluid. In a retrospective study of 108 patients who underwent revision TKA, the LE test was 80.6% sensitive and 100% specific [28]. However, the presence of blood and blood debris, in the synovial fluid aspirates, may negatively affect this colorimetric test [29]. A simple solution to this problem is the use of a centrifuge for blood contaminated joint aspirations which was found not to alter the accuracy of the LE test [30]. The use of the LE test has been recently validated and LE was adopted as a minor criterion in the definition of PJI according to the ICG [6, 31].
- ***Human α-Defensin*** is released by local neutrophils and is part of an innate immune response to infection. It is an antimicrobial peptide that acts *via* the insertion of voltage sensitive channels into the bacterial membrane [34]. A few recent studies have advocated the use of synovial α-defensin for the diagnosis of PJI; and this marker better aligns with the MSIS criteria compared with other tests that are routinely used for the diagnosis of PJI (culture, ESR, CRP, synovial WBC count, and LE) [35 - 37]. Nonetheless, as mentioned earlier, the test performed considerably better when it was combined with the synovial CRP levels [36].

Among inflammatory cytokines, IL-6 in particular has been the focus of many studies and one study found that synovial fluid IL-6 outperformed serum IL-6 and serum CRP [38]. Interestingly, the predictive power of diagnosing a PJI was increased with combination of both serum and synovial fluid IL-6, compared with performing each test individually. The same study showed that serum IL-6 was associated with significantly higher values in the PJI group as

compared to the aseptic loosening group, with specificity at 58.3% at a cut-off value of 2.6 pg/ml and that with a cut-off >6.6 pg/ml, the specificity increased to 88.3% [38]. Similarly, synovial fluid IL-6 with a cut-off of >2100 pg/ml had a specificity of 85.7% and at levels >9000 pg/ml, specificity was almost at 100%, so PJI could be considered proven with IL-6 levels above this threshold [38]. Other studies found that synovial IL-6 not only had high specificity and accuracy, but it also had significantly higher accuracy than the current standard tests for PJI, even with the inclusion of patients who were taking antibiotics and those with systemic inflammatory diseases [23, 25]. While there are many synovial fluid biomarkers that could assist in the diagnosis of PJI, these markers must be studied further before they can be added to the surgeon's armamentarium for diagnosing PJI.

Toll-like receptors (TLR) are transmembrane receptors that recognize pathogen-associated molecular patterns (PAMPs) and play an integral role in the activation of host inflammatory response against microbial infections [39]. Certain TLRs, including TLR-1 and TLR-6, are activated by bacterial lipoproteins, which make them candidates as good biomarkers for diagnosis of PJI [40, 41]. A pilot study of 55 patients who underwent revision total joint arthroplasty found that TLR-1 and TLR-6 were significantly elevated in the tissue of patients who had a PJI compared with those who were aseptic. Both TLR-1 and TLR-6 had high specificity and sensitivity for diagnosing PJI, with TLR-1 outperforming TLR-6 with a specificity of 100% and sensitivity of 95%. The main disadvantage of this method is the time that is required to process the periprosthetic tissue for RNA extraction and for real-time polymerase chain reaction (PCR). Hence, in an attempt to increase the practicality of using TLR levels as a method to detect and guide treatment of PJI, RNA isolates from synovial fluid neutrophils are currently being studied [41].

DIAGNOSTIC METHODS TARGETING BIOFILM

The biofilm theory of bacterial growth is considered to have a major role in the pathogenesis of PJI [42, 43]. Moreover, many challenging aspects of the prevention, diagnosis, and eradication of PJI can be explained with this theory. Implants provide a platform for the initial adherence and multiplication of bacteria. Biofilm consists of a complex matrix of extracellular polymers (such as polysaccharides, glycoproteins, and DNA) in which bacteria can be protected from the host immune response and antimicrobial agents. Therefore, a minimal inflammatory response is elicited despite the presence of abundant bacteria on the prosthesis. This allows the bacteria to survive and grow on the surface of the prosthesis without being affected by the environment outside the biofilm. Moreover, bacteria lodged on the biofilm can stay in a metabolically quiescent state and be responsible for culture-negative and antibiotic-resistant PJI [44]. Therefore, diagnostic methods that aim to target biofilm components (extracellular molecules or lodged bacteria) can improve our ability to diagnose PJI.

Methods to Improve the Sensitivity of Cultures

Conventional microbiological culture methods have several limitations including risk of false-positive (contamination) results and their inability to isolate the micro-organisms, *i.e.* culture-negative infections. Therefore, improving culture methods has been an area of interest for more accurate diagnosis of PJI [45]. Sonication of explanted components improves the yield of the bacterial mass attached to the implants and therefore increases the sensitivity and specificity of conventional culture techniques [46], even in patients who are already receiving antibiotic therapy [47]. Furthermore, the use of sonication in combination with other diagnostic techniques, such as multiplex PCR, can improve the identification of bacteria compared with conventional methods [48, 49]. Additionally, more bacterial pathogens are identified through the incubation of the samples obtained *via* sonication into specific culture media, such as enriched blood culture media, compared to the incubation of synovial fluid in enriched blood culture media [50]. Other studies have reported that using enriched blood culture media considerably decreased the time required for cultivation of bacteria, with the majority of organisms growing within only 3 days [51].

Biofilm Visualization and Sequencing-based Biomolecular Methods

The biofilm and its physical structure can be visualized with the use of fluorescence *in situ* hybridization (FISH), which uses fluorescent DNA or peptide nucleic acid probes to bind to specific targets in the bacteria, including ribosomal RNA and the genes responsible for antibiotic resistance [43, 52]. The amount of a specimen that FISH can analyze at one time is low, and similar to PCR, FISH is limited by probe selection. Nevertheless, the risk of false-positive rates is lower than PCR and if FISH is combined with viability staining methods it can be optimized to measure only live bacteria. Similarly, customized grids of DNA microarrays consisting of thousands of probes for ribosomal or antibiotic-resistance genes of the most common PJI pathogens can be fabricated to capture fluorescently labeled DNA in clinical samples. This strategy can decrease the cost and improve the time-efficiency of FISH and PCR [53].

PCR-based Electron Spray Ionization Time-of-flight Mass Spectrometry (ESI-TOF-MS)

The identification of specific pathogens through molecular techniques with the use of PCR-based assays was originally studied because standard cultures failed to identify organisms that caused an infection. Earlier PCR-based assays used species-specific primers or a single pan-domain primer pair for the 16S ribosomal RNA gene, but they led to a higher rate of false-positives due to contamination and higher false-negatives because the probes could not cover the wide spectrum of pathogens responsible for infection. The Ibis T5000 biosensor system is a novel technology that uses a pan-domain DNA-based amplification technique to improve the utility of PCR in diagnosing PJI [54]. PCR is used to amplify the DNA samples with multiple different primers, and the resulting PCR amplicons are sequentially electron sprayed into a time-of-flight mass spectrometer. The spectral signals from the mass spectrometer are used to determine the mass of each PCR amplicon, which can be used to identify the bacterial species that are present in the sample [54, 55]. Ibis T5000 was not only able to verify positive conventional culture results, but was also able to detect an organism in four out of five cases of PJI that was thought to be culture-negative. Additionally, Ibis found that 88% of the revision cases that were presumed aseptic were actually cases that had a subclinical infection [54].

Matrix-assisted Laser Desorption Ionization Time-of-flight Mass Spectrometry (MALDI-TOF/MS)

This novel proteomic technology identifies bacteria *via* analysis of their macromolecular profile. Laser ionization is used to measure the charge and molecular mass of the bacterial surface proteins. Since individual bacterial species have a unique mass-to-charge ratio, the obtained information is cross-matched with a bacterial spectra database (such as MALDI Bio-typer database) to identify the causative pathogen for PJI [56, 57]. This method is rapid and cost-effective, and has been performed on different bodily fluids (including periprosthetic joint fluid) with high agreement compared with standard methods for bacterial identification [58, 59].

CONCLUSION

The current tests that are available to diagnose PJI have considerably improved. With the use of novel approaches such as metabolomics and proteomics, biomarkers can be found and used to diagnose infection in its early stages. Furthermore, new techniques to disrupt biofilms, microbiological processes such as beadmill processing [60], and quantitative molecular methods can be used to improve the accuracy of identifying pathogens. With these advances, rapid, precise, and cost-effective methods will be used to diagnose PJI and help guide treatment for this devastating complication of total joint arthroplasty.

CONFLICT OF INTEREST

JP is an equity owner in CD Diagnostics, a company that is involved in developing molecular biomarker for diagnosis of PJI. JP is also a paid consultant to various companies that are involved in development of novel techniques for management of PJI.

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