Review Paper

The Role of Synovial Cytokines in the Diagnosis of Periprosthetic Joint Infections: Current Concepts

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Abstract

Periprosthetic joint infection (PJI) is a serious and potentially devastating complication of total joint arthroplasty. Accurate diagnosis of PJI is of utmost importance, but differentiating septic from aseptic failed total joint arthroplasty is extremely challenging, and improper management can lead to significant morbidity. The gold standard for PJI diagnosis is based on standardized laboratory and clinical criteria but relies on preoperative and intraoperative findings, which can be unreliable. Given these limitations, research has focused on new methods for diagnosing PJI. Synovial fluid inflammatory cytokines have been found to accurately diagnose PJI. In this article, we review the synovial fluid cytokines that are being used as aids in PJI diagnosis.

Total joint arthroplasty (TJA) is an effective procedure that has been extensively used to relieve pain and improve quality of life in patients with various forms of joint disease. Although advances in technology and surgical technique have improved the success of TJA, periprosthetic joint infection (PJI) remains a serious complication. In the United States, it is estimated that PJI is the most common reason for total knee arthroplasty failure and the third most common reason for total hip arthroplasty revision.[1] Although the incidence of PJI is 1% to 2%, the dramatic increase in TJA volume is expected to be accompanied by a similar rise in the number of infected TJAs; that number is expected to exceed 60,000 in the United States by 2020.[2] Moreover, management of PJI is expensive and imposes a heavy burden on the healthcare system, with costs expected to hit $20 billion by 2020 in the US.[2] Therefore, treating asepsis cases as infections imposes a heavy burden on the healthcare system and may result in excessive morbidity.[3] At the same time, inadequate management of a PJI may result in recurrences that require infection treatment with morbid procedures, such as arthrodesis or amputation. Accurate diagnosis of PJI is of paramount importance in preventing potential implications of a misdiagnosed case. Unfortunately, the PJI diagnosis is extremely challenging, and the available diagnostic tests are often unreliable.[4] Thus, research has recently focused on use of several synovial fluid cytokines in the detection of PJI.[5-7] In this article, we provide an overview of the synovial biomarkers being used to diagnose PJI.

Diagnosis of Periprosthetic Joint Infection

Differentiating between septic and aseptic failed TJA is important, as the treatment options differ considerably. PJI can be broadly classified as acute or early postoperative (<6 weeks), late chronic (indolent onset), and acute-on-chronic (acute onset in well-functioning prosthesis, secondary to hematogenous spread).[8] The acute and acute-on-chronic presentations are often associated with obvious signs of infection.[9] However,

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Table 1. **Periprosthetic Joint Infection, Defined by International Consensus Group**

Major Criteria	Minor Criteria
Two positive periprosthetic cultures with phenotypically identical organisms	Elevated serum C-reactive protein and elevated erythrocyte sedimentation rate
Sinus tract communicating with joint	Elevated synovial fluid white blood cell count or ++ change on leukocyte esterase test strip
	Elevated synovial fluid polymorphonuclear neutrophil percentage
	Positive histologic analysis of periprosthetic tissue
	Single positive culture

chronic and low-grade infections pose a challenge to modern orthopedic practice, as the symptoms often overlap with that of aseptic causes of TJA failure.[10] As a result, the International Consensus Group on Periprosthetic Joint Infection developed complex criteria using the Musculoskeletal Infection Society definition of PJI and involving a battery of tests for PJI diagnosis.[11] According to these criteria, PJI is diagnosed when 1 of the 2 major criteria or 3 of the 5 minor criteria are met (**Table 1**).

Although these criteria constitute the most agreed on and widely used standard for PJI diagnosis, the definition is complex and often incomplete until surgical intervention. An ideal diagnostic test would aid in managing a PJI and provide results before a treatment decision is made. Many revision surgeries are being performed with insufficient information about the true diagnosis, and the diagnosis might change during or after surgery. About 10% of the revisions presumed to be aseptic may unexpectedly grow cultures during surgery and thereby satisfy the criteria for PJI after surgery.[12] Moreover, with the use of novel methods such as polymerase chain reaction, microorganisms were identified in more than three-fourths of the presumed aseptic revisions.[13] The optimal management of such cases is controversial, and it is unclear whether positive cultures should be treated as possible contaminants or true infection.[12,14]

Verification of Infection Eradication

A 2-stage revision procedure, widely accepted as the standard treatment for PJI, has success rates approaching 94%.[15] In this procedure, it is important to verify infection eradication before beginning the second-stage reimplantation. Verification is crucial in avoiding reimplantation of an infected joint.[16] After the first stage, patients are usually administered intravenous antibiotics for at least 6 weeks; these antibiotics are then withheld, and systemic inflammatory markers are evaluated for infection eradication. Although reliable criteria have been established for PJI diagnosis, guidelines for detecting eradication of infection are rudimentary. Most surgeons monitor the decrease in serologic markers, such as erythrocyte sedimentation rate and C-reactive protein (CRP) level, to assess the response to treatment. However, noninfectious etiologies may result in continued elevation of these markers.[17] Even though aspirations are often performed to diagnose persistent infection before the second-stage procedure, their diagnostic utility may be limited.[18] Use of cultures is also limited, as presence of antibiotic-loaded spacers can decrease the sensitivity of culture.[19] Inadequate diagnosis often leads to unnecessary continuation of antimicrobial therapy or additional surgical débridement. Nuclear scans often remain positive because of aseptic inflammation related to surgery and are not useful in documenting sepsis arrest.[20] Given the limitations of available tests, novel strategies for identifying the presence of infection at the second stage are being tested.

Synovial Fluid Cytokines

PJI pathogenesis begins with colonization of the implant surfaces with microorganisms and subsequent formation of biofilms.[21] The

Take-Home Points

- In cases of failed TJA, it is important to differentiate between septic and aseptic etiologies.
- Chronic and low-grade infections are challenging for orthopedic surgeons, as the symptoms often overlap with aseptic etiologies.
- Verification of infection eradication before beginning the second-stage reimplantation surgery is extremely important, but pre- and intraoperative findings can be unreliable.
- Synovial fluid cytokines have been shown to accurately diagnose PJIs.
- Synovial fluid cytokines may help surgeons differentiate between septic and aseptic cases of failed TJA.

human immune system is activated by the microbial products, cell wall components, and various biofilm proteins. Immune cells are recruited to the site, where they secrete a myriad of inflammatory biomarkers, such as cytokines, which promote further recruitment of inflammatory cells and aid in the eradication of pathogens.[9] These inflammatory cytokines and cells are involved in aseptic inflammatory joint conditions, such as rheumatoid arthritis[22,23]; however, some are specifically involved in immune pathways combating pathogens.[24] This action is the basis for increasing interest in using various synovial fluid cytokines and other biomarkers in the diagnosis of PJI. Here we describe some of the commonly studied cytokines.

Interleukin 1β

Interleukin 1β (IL-1β) is a major proinflammatory cytokine that is synthesized by multiple cells, including macrophages and monocytes.[25] IL-1β is produced in response to microorganisms, other cytokines, antigen-presenting cells, and immune complexes; stimulates production of acute-phase proteins by the liver; and is an important pyrogen.[25] Deirmengian and colleagues[5] found that synovial IL-1β increased 258-fold in patients with a PJI. Studies have found that synovial IL-1β has sensitivity ranging from 66.7% to 100% and specificity ranging from 87% to 100%, with 1 study reporting an accuracy of 100%.[5,6,26,27]

Interleukin 6

Also produced by macrophages and monocytes, interleukin 6 (IL-6) is a potent stimulator of acute-phase proteins.[28,29] IL-6 has a role as a chemoattractant and helps with cell differentiation when changing from innate to acquired immunity.[30] It is also used as an aid in diagnosing PJI; it has sensitivity ranging from 62% to 100% and specificity ranging from 85% to 100%.[5,6,26,31,32] Synovial IL-6 measurements were more accurate than serum IL-6 measurements.[26] Furthermore, synovial IL-6 can be increased up to 27-fold in PJI cases.[5] In one study, synovial IL-6 levels >2100 pg/mL had sensitivity of 62.5% and specificity of 85.7% in PJI diagnosis[26]; in another study, an IL-6 threshold of 4270 pg/mL had sensitivity of 87.1%, specificity of 100%, and accuracy of 94.6%.[31]

C-Reactive Protein

CRP is an acute-phase reactant. Blood levels increase in response to aseptic inflammatory processes and systemic infection.[33] CRP plays an important role in host defense by activating complement and helping mediate phagocytosis.[33,34] Although serum CRP levels have been used in diagnosing PJIs,[6] they can yield false-negative results.[35,36] Therefore, attention turned to synovial CRP levels, which were found to be increased 13-fold in PJI cases.[5] It has been shown that synovial CRP levels are significantly higher in infected vs noninfected prosthetic joints[34] and had diagnostic accuracy better than that of serum CRP levels in diagnosing PJI.[37] One study found that CRP at a threshold of 3.7 mg/L had sensitivity of 84%, specificity of 97.1%, and accuracy of 91.5%,[37] whereas another study found that CRP at a threshold of 3.61 mg/L had sensitivity of 87.1%, specificity of 97.7%, and accuracy of 93.3%.[31]

α-Defensin

α-Defensin, a natural peptide produced and secreted by neutrophils in response to pathogens, has antimicrobial and cytotoxic properties,[38-40] signals for the secretion of various cytokines, and acts as a chemoattractant for various immune cells.[41] Deirmengian and colleagues[6] found that α-defensin was consistently elevated in patients with PJI. α-Defensin is extremely accurate in diagnosing PJI; it has sensitivity ranging from 97% to 100% and specificity ranging from 96% to 100%.[6,27,42] Moreover, α-defensin was effective in diagnosing PJI caused by a wide spectrum of organisms, including various low-virulence bacteria and fungi.[43]

Leukocyte Esterase

Leukocyte esterase is an enzyme produced and secreted by neutrophils at sites of active infection.[7,44] Testing for this enzyme is performed with a colorimetric strip and was originally performed for the diagnosis of urinary tract infections.[44,45] In a study conducted by Parvizi and colleagues,[7] this strip was used to test for leukocyte esterase in synovial fluid samples; a ++ reading was found to have sensitivity of 80.6% and specificity of 100% in diagnosing knee PJI. Similarly, De Vecchi and colleagues[45] found sensitivity of 92.6% and specificity of 97%.

Other Synovial Markers

Research has identified numerous molecular biomarkers that may be associated with the pathogenesis of PJI. Although several (eg, cytokines) have demonstrated higher levels in synovial fluid in patients with PJI than in normal controls, only a few have had clinically relevant diagnostic

Table 2. **Most Widely Studied Synovial Fluid Biomarkers**

Synovial Cytokine	Source	Sensitivity, %	Specificity, %
Interleukin 1β[5,6,26,27,50]	Primarily produced by local macrophages	66.7-100	87-100
Interleukin 6[5,6,26,31,32]	Predominantly from inflammatory cells; also produced by synovium and, to lesser extent, chondrocytes	62-100	85-100
C-reactive protein[6,31,37,52,53]	Acute-phase reactant produced by hepatocytes	84-95	85-97
α-Defensin[6,27,42]	Antimicrobial peptides secreted by cells of innate immunity, primarily neutrophils	97-100	96-100
Leukocyte esterase[44,45,54]	Marker of white blood cells; detects hydrolytic enzyme esterase produced by white blood cells	79-95	73-97

utility.[6] Deirmengian and colleagues[6] screened 43 synovial fluid biomarkers that potentially could be used in the diagnosis of PJI. Besides the cytokine α-defensin, 4 other biomarkers—lactoferrin, neutrophil gelatinase-associated lipocalcin, neutrophil elastase 2, and bactericidal/permeability-increasing protein—had accuracy of 100%. In addition, 8 cytokines and biomarkers (IL-8, CRP, resistin, thrombospondin, IL-1β, IL-6, IL-10, IL-1α) had area under the curve values higher than 0.9. Studies have also evaluated the diagnostic utility of metabolic products such as lactate, lactate dehydrogenase, and glucose; their accuracy was comparable to that of serum CRP.[32]

Serum Markers

In addition to the synovial fluid cytokines, several serum inflammatory cytokines have been studied as potential targets in diagnosing infection. Serum IL-6 has had excellent diagnostic accuracy[46] and, when combined with CRP, could increase sensitivity in diagnosing PJI; such a combination (vs either test alone) could be useful in screening patients.[47,48] Biomarkers such as tumor necrosis factor α and procalcitonin are considered very specific for PJI and may be useful in confirmatory testing.[48] Evidence also suggests that toll-like receptor 2 proteins are elevated in the serum of patients with PJI and therefore are a potential diagnostic tool.[49]

Limitations of Synovial Cytokines

The literature suggests that some synovial fluid cytokines have promise.[6] However, the best biomarker or combination of biomarkers is yet to be determined. Results have been consistent with α-defensin and other cytokines but mixed with IL-6 and still others[32,42,50] (**Table 2**). In addition, the techniques for detecting these biomarkers are not fully standardized, limiting their generalizability. PJI diagnostic tests based on biomarkers are expensive, require special expertise, and are limited to only a few centers. Apart from synovial leukocyte esterase, none of the newly investigated biomarkers are included in current guidelines.[11] Given the lack of consensus and guidelines, biomarkers are rarely used to guide treatment decisions. However, with the increase in supportive evidence, incorporation of biomarkers into the general PJI guidelines is expected.

Information on the utility of synovial biomarkers in detecting persistent infection is limited. Frangiamore and colleagues[50] found that IL-1 and IL-6 levels decreased between the stages of 2-stage revision. Unfortunately, none of the synovial fluid cytokines investigated (IL-1, IL-2, IL-6, IL-8, Il-10, interferon γ, granulocyte macrophage-colony stimulating factor, tumor necrosis factor α, IL-12p70) satisfactorily detected resolution of infection in the setting of prior treatment for PJI. Although cytokines are expected to be elevated in the presence of infection, the internal milieu at the time of stage 2 of the revision makes diagnosis of infection difficult. In addition, presence of spacer particles and recent surgery may activate immune pathways and yield false-positive results. Furthermore, antibiotic cement spacers may suppress the microorganisms to very low levels and yield false-negative results even if these organisms remain virulent.[19]

Even though the synovial molecular markers can detect the presence of infection, they are unable to identify pathogens. As identifying the pathogen is important in the treatment of PJI, there has been interest in using polymerase chain reaction (PCR) techniques.[51] These tests may also provide specific information about the pathogen, such as its antibiotic sensitivity. A recently developed technology, the Ibis T5000 Universal Biosensor

(Ibis Biosciences), uses novel pan-domain primers in a series of PCRs. This biosensor is useful in diagnosing infections when cultures are negative and appears to be more accurate than conventional PCR.[13] As reported by Jacovides and colleagues,[13] this novel PCR technique identified an organism in about 88% of presumed cases of aseptic revision.

Conclusion

PJI poses an extreme challenge to the healthcare system. Given the morbidity associated with improper management of PJI, accurate diagnosis is of paramount importance. Given the limitations of current tests, synovial fluid cytokines hold promise in the diagnosis of PJIs. However, these cytokines are expensive, and their clinical utility in PJI management is not well established. More research is needed before guidelines for synovial fluid cytokines and biomarkers can replace or be incorporated into guidelines for the treatment of PJIs.

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