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The role of biomarkers in the diagnosis of periprosthetic joint infection

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- The role of serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as the first line for evaluating a patient with periprosthetic joint infection (PJI) has been debunked.
- We are living in the era of biomarkers for the diagnosis of PJI, and to that effect, several biomarkers have been introduced such as synovial fluid alpha defensin and leukocyte esterase.
- The synovial fluid leukocyte esterase test has a low cost, is accessible, and has provided promising results for diagnosing PJI.
- There is an urgent need for an accurate and reliable serum biomarker for diagnosing patients with PJI.

Keywords: diagnosis; periprosthetic joint infection; PJI; serum biomarkers; synovial fluid biomarkers; total joint arthroplasty

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Introduction

Periprosthetic joint infection (PJI) is responsible for 25% of failed total knee arthroplasties[1] and 15% of revision total hip arthroplasties.[2] PJI has a huge economic burden on the health care system, and there will be a substantial increase in the number of patients who are diagnosed with PJI in the years to come due to the increasing volume of total joint arthroplasties (TJAs) performed internationally.[3,4] In assessing a painful joint after TJA, it is critical for the surgeon to distinguish between septic and aseptic failure, as the treatment protocol for PJI mandates specific surgical strategies that aim to eliminate the infecting microorganism(s).[5-8]

Diagnosis of PJI is challenging as there is no absolute test to confirm or exclude infection. Hence, the clinician has to use a combination of tests, all of which (besides their expense) can be invasive and are not absolutely accurate.[9]

There is emerging evidence that a host with an infected joint is likely to mount a primitive, but specific, innate immune response to the pathogens in the infected joint.[10-14] This innate immune response is responsible for triggering the systemic immune system and a cascade of protective pathways in the host. Microarray techniques have shown a specific gene expression signature exhibited by the white blood cells (WBCs) in the synovial fluid of infected joints, distinctive of the innate host immune response to infection.[10] This unique response was also observed at the level of the proteome, revealing several biomarkers that can potentially be used for diagnosing PJI; interestingly enough, many of these biomarkers diagnostically outperform the currently available tests for PJI.[15,16] In 2013, our group evaluated 16 promising synovial fluid biomarkers for the diagnosis of PJI and provided the sensitivity and specificity of each biomarker (Table 1).

In this review, we aimed to analyse the current diagnostic measures for PJI, with a special focus on molecular biomarkers.

Serum biomarkers

Serum markers are favourable diagnostic tools due to the ease of taking blood and its low-risk nature, compared with synovial fluid aspiration. The American Academy of Orthopaedic Surgeons and the International Consensus meeting on PJI currently recommend the assessment of patients' serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as the first line of diagnostic evaluation in patients with suspected PJI. Reports have shown a sensitivity of 91% and specificity of 72% for ESR and a sensitivity of 94% and specificity of 74% for CRP.[7,9,17] ESR and CRP are well-known biomarkers of systemic responses to inflammation.[18]

However, there are issues with the serum markers of inflammation, namely CRP and ESR. These markers are elevated with any type of inflammation and infection, compromising their specificity for diagnosis of PJI. Recent evidence suggests that PJI with some slow-growing organism may not result in a florid physiological response and hence may not result in elevation of ESR and CRP in the serum, raising a concern regarding the sensitivity of the tests in some settings. In addition, a recent study from our

Table 1. Evaluation of promising synovial fluid biomarkers for the diagnosis of periprosthetic joint infection[43]

Biomarker	AUC	Cut-off	Specificity (%)	95% CI (%)	Sensitivity (%)	95% CI (%)
α-Defensin	1.000	4.8 μg/mL	100	95–100	100	88–100
ELA-2	1.000	2.0 μg/mL	100	95–100	100	88–100
BPI	1.000	2.2 μg/mL	100	95–100	100	88–100
NGAL	1.000	2.2 μg/mL	100	95–100	100	88–100
Lactoferrin	1.000	7.5 μg/mL	100	95–100	100	88–100
IL-8	0.992	6.5 ng/mL	95	87–99	100	87–100
SF CRP	0.987	12.2 mg/L	97	90–100	90	73–98
Resistin	0.983	340 ng/mL	100	95–100	97	82–99
Thrombospondin	0.974	1061 ng/mL	97	90–100	90	73–98
IL-1β	0.966	3.1 pg/mL	95	87–99	96	82–100
IL-6	0.950	2.3 ng/mL	97	89–100	89	71–98
IL-10	0.930	32.0 pg/mL	89	79–96	89	72–98
IL-1α	0.922	4.0 pg/mL	91	81–97	82	63–94
IL-17	0.892	3.1 pg/mL	99	92–100	82	63–94
G-CSF	0.859	15.4 pg/mL	92.	82–97	82	62–94
VEGF	0.850	2.3 ng/mL	77	65–87	75	55–89

AUC = area under the curve; α-defensin = human α-defensin 1-3; ELA-2 = neutrophil elastase 2; BPI = bactericidal/permeability-increasing protein; NGAL = neutrophil gelatinase-associated lipocalin; SF = synovial fluid; CRP = C-reactive protein; G-CSF = granulocyte colony-stimulating factor; VEGF = vascular endothelial growth factor.

institution has shown that the administration of systemic antibiotics to patients with PJI may compromise the results of these laboratory values.[19]

It is also imperative for clinicians to consider the timing of infection prior to assessing patients' ESR and CRP results, as they are usually elevated in the early post-operative period. ESR can be elevated for up to six weeks after surgery, and CRP by up to 2 weeks post-surgery.[20] Therefore, the use of ESR and CRP for diagnosis of PJI is only meaningful when the other Musculoskeletal Infection Society (MSIS) diagnostic criteria are present.

Other serum biomarkers

There is a dire need of a serum biomarker for diagnosing PJI and numerous efforts have been made to pursue this goal.

Procalcitonin (PCT) is a serum biomarker that is elevated in the presence of bacteria. One study[21] measured serum levels of PCT, interleukin (IL)-6, tumor necrosis factor (TNF)-α, ESR, and CRP in 78 patients undergoing revision total arthroplasty for sepsis. The sensitivity of CRP and IL-6 were highest (95%) for diagnosing PJI when the levels were greater than 3.2 mg/dL and 12 pg/mL respectively. The authors recommended the combination of CRP and IL-6 as a screening test for PJI. PCT levels (> 0.3 ng/mL) were very specific (98%), but had a low sensitivity (33%).

On the contrary, Hügle et al[22] showed that PCT with a threshold of 0.25 ng/mL has a higher sensitivity and specificity for diagnosing septic arthritis than CRP, with a sensitivity of 93% and specificity of 75%. This can possibly be explained by the fact that PCT is secreted by the mononuclear phagocyte system when stimulated by lipopolysaccharide. Therefore, PCT can be a useful tool to differentiate between bacterial infections of the joint and other causes of inflammation.

However, more recent studies have claimed that PCT is not a very accurate tool for diagnosing PJI.[23,24] In these studies, all patients with septic loosening had an increased serum ESR, CRP, WBC, IL-6, soluble intercellular adhesion molecule-1, and serum IgG to short-chain exocellular lipoteichoic acid. IL-6 is secreted by different immune cells and triggers the excretion of CRP; therefore, it is believed that the IL-6 level rises much faster than CRP and has been reported to be a sensitive marker for diagnosing PJI.[21,25]

Shah et al[26] measured the levels of 25 different serum cytokines before and after TJA. Of the measured cytokines, IL-6, monocyte chemoattractant protein (MCP)-1 and IL-2R were associated with post-surgical trauma in one PJI patient. The authors concluded that these serum markers can be helpful for the early post-operative diagnosis of PJI. Wirtz et al[27] also advocated the role of IL-6, and in their prospective study showed that it is a better indicator for post-operative inflammatory response than CRP in patients undergoing TJA.

Synovial fluid biomarkers

Synovial fluid biomarkers play a very important role in the diagnosis of PJI. They can be categorised into two main groups: cytokines and biomarkers with antimicrobial functions.[10] When infection occurs in a joint, cytokines such as IL-1b, IL-6, IL-8, IL-17, and TNF-α are released from macrophages.[15,28] Studies have shown that vascular endothelial growth factor, which is a marker for angiogenesis, also increases in PJI patients.[16] The problem with this group of biomarkers is the low specificity, and these markers can be elevated in other inflammatory conditions of the joint such as rheumatoid arthritis.

More specific synovial fluid biomarkers are: leukocyte esterase (LE), human α-defensin, human β-defensin synovial CRP, and cathelicidin LL-37. Leukocyte esterase is an enzyme that is secreted by the activated neutrophils, and has been utilised in other types of infection, especially

urological conditions. In the setting of PJI, neutrophils that are recruited to the joint secrete LE that can be detected using colourimetric strip tests via reactions that result in a colour change.[29] Leukocyte esterase is a simple, readily available test, requiring application of synovial fluid to a urine test strip. It is now part of the minor criteria of the MSIS diagnostic criteria for PJI.[30] Tischler et al[31] demonstrated that the LE strip test has a high specificity, positive predictive value, negative predictive value, and moderate sensitivity for diagnosing PJI. Wetters et al[32] investigated the accuracy of the LE test and reported a sensitivity of between 92.9% and 100%, and a specificity between 77.0% and 88.8%. Only non-blood-contaminated samples can be evaluated for the LE test, as the presence of blood can potentially interfere with the colourimetric changes of the test strip.[31]

The synovial fluid α-defensin test has shown promising results, with a sensitivity of 97% and a specificity of 96% for diagnosing PJI.[33] Defensins are 2-6 kDa cationic microbicidal peptides that are active against many Gram-negative and Gram-positive bacteria, fungi, and enveloped viruses.[34] Defensins in mammals are classified into alpha, beta and theta categories, based on their size and pattern of disulfide bonding. Alpha-defensins are particularly found in neutrophils, certain macrophage populations and Paneth cells. Defensins are produced in response to microbial products or pro-inflammatory cytokines.

The α-defensin mechanism by which micro-organisms are destroyed and inactivated is not yet fully understood. Nevertheless, the general belief is that the destruction is a consequence of disruption to the micro-organism's membrane.[35] The spatially separated, charged, and hydrophobic regions, along with the polar topology of α-defensin, allows it to insert itself into the membranes; therefore, the hydrophobic regions are buried within the phospholipid membrane interior, and the cationic sites interact with anionic phospholipid head groups and water. The disruption of membrane integrity and function leads to lysis of the micro-organisms.[36] In other words, defensins, especially α-defensin, are from primitive immune systems that are innately activated and function locally regardless of the systematic response.[37]

Several studies have endorsed the role of the α-defensin test in diagnosing patients with PJI. The α-defensin test provides consistent results regardless of the organism type, Gram staining, species, or virulence of the organism, and should be seriously considered as a standard diagnostic tool in the evaluation for PJI.[10] In another study by Bingham et al,[38] the authors concluded that the sensitivity and specificity of the synovial fluid α-defensin assay exceeded the sensitivity and specificity of other currently available clinical tests.

CRP, which is elevated in both the serum and synovial fluid of PJI cases, is a protein that is synthesised in the liver in response to acute inflammation when there are increased macrophages.[39] Parvizi et al[40] found a statistically significant difference in the mean of synovial fluid CRP comparing septic with aseptic patients. There was a mean of 40 mg/L *vs* a mean of 2 mg/L, respectively ($p < .0001$). The study found a sensitivity of 85% and a specificity of 95% when 9.5 mg/L was considered the threshold.

Human host defense peptide LL-37 is one of the cathelicidins and is an antimicrobial peptide that induces mediators such as IL-8, and regulates the inflammatory response.[41,42] Gollwitzer et al[28] demonstrated that LL-37 was elevated in the synovial fluid of PJI patients and reported a sensitivity of 80% and specificity of 85%, with an area under the curve of 0.875.

Conclusion

Modern medicine has entered a new era where molecular biomarkers play an increasingly important role in the diagnosis of various conditions. Biomarkers also hold great promise for diagnosis of PJI. However, efforts must continue to find cost-effective and accessible biomarkers, preferably measured in the serum, with all their potential benefits.

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CONFLICT OF INTEREST

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