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# The role of alpha defensin in prosthetic joint infection (PJI) diagnosis: a literature review

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- Prosthetic joint infection (PJI) management is not standardized worldwide and the outcome is frequently unsatisfactory.
- More and more arthroplasties are now being performed. An increasing number of highly virulent and antibiotic-resistant bacteria and an ageing population of patients presenting with many comorbidities make it necessary to focus on this important topic.
- Diagnosis of PJI remains challenging because the clinical signs and symptoms and elevation of systemic biomarkers (C-reactive protein, erythrocyte sedimentation rate) may be unclear.
- In the last few years, the clinical research has focused on synovial fluid biomarkers as a possible breakthrough in the complex scenario of PJI diagnosis.
- Synovial biomarkers have shown encouraging results and they should be used as diagnostic adjuncts to synovial white cell count and culture bacteriology. Synovial leukocyte esterase (LE) and synovial C-reactive protein (CRP) have been evaluated as good screening measures; however, the most promising synovial fluid biomarker in terms of sensitivity and specificity for PJI seems to be alpha defensin (AD).
- The laboratory-based alpha defensin enzyme-linked immunosorbent assay (ELISA) test demonstrated the highest ever reported accuracy for PJI diagnosis. However, an alpha defensin lateral flow test could have its place in ruling in a suspected PJI intraoperatively because of its high specificity and rapid results.

**Keywords:** alpha defensin; PJI; prosthetic joint infections; synovial biomarkers

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## Introduction

Prosthetic joint infections (PJI) occur in 0.7% to 2.4% of patients and are responsible for 15% of failed total hip arthroplasties and 25% of revision total knee arthroplasties.[1,2] Almost any microorganism can cause PJI, such as Gram-positive bacteria (accounting for about two-thirds of the total number of infections), Gram-negative bacteria and polymicrobic flora (accounting for about 10–15% of infections each), and fungi (rare).[3–5] The management of infections is not standardized worldwide and the outcome is frequently unsatisfactory because of the increasing number of highly virulent and antibiotic-resistant bacteria, and due to an ageing patient population presenting with many comorbidities.

PJI diagnosis is challenging because clinical signs and symptoms and systemic biomarker elevation (CRP, ESR) may be unclear in the most frequent delayed, low-grade and/or late infections, and in patients who have undergone previous/concomitant antibiotic therapy. Frozen sections are not routinely performed in hospitals, and synovial fluid white blood cell count and differential white blood cell count, while easy to collect in the case of the knee, are sometimes difficult or unreachable in the hip.[6–12] Moreover, metallosis and other chronic inflammatory diseases can mimic the clinical and biochemical picture of PJI.

## PJI diagnosis

In 2011, in an attempt to guide clinicians in everyday practice, the Musculoskeletal Infection Society (MSIS) published a diagnostic approach which includes two major or six minor criteria for diagnosis of PJI, where the presence of either one of the major or at least four of the minor criteria would indicate PJI.[13] In 2013 the International Consensus Group on Periprosthetic Joint Infection

held in Philadelphia slightly modified the MSIS criteria. The purulence of synovial fluid was removed as a minor criterion while the leukocyte esterase test was added as an alternative option to assess elevation of synovial fluid white blood cell (WBC) count. The consensus group also determined different thresholds for the minor criteria, acceptable for both hip and knee replacements, based on the acuity of the infections. According to the PJI Consensus Group, patients should be considered to have PJI if they meet one of the major criteria or at least three of the minor criteria.[14] In the 2018 definition of periprosthetic hip and knee infection,[28] new diagnostic tests that allow surgeons to reach a preoperative diagnosis finally found their place.

## Biomarkers

In the last few years, the clinical research has focused on synovial fluid biomarkers as a possible breakthrough in the complex scenario of PJI diagnosis. Numerous biomarkers have been evaluated and become available[15,29,30] including synovial leukocyte esterase (LE),[19,31,32] synovial alpha defensin (AD),[16,33] and synovial C-reactive protein (CRP).[34,35]

Deirmengian et al[15] have identified and studied the diagnostic characteristics of 16 promising synovial fluid biomarkers for PJI diagnosis. The biomarkers under investigation were: alpha defensin (AD), IL-1a, IL-1, IL-6, IL-8, IL-10, IL-17, granulocyte colony-stimulating factor (G-CSF), vascular endothelial growth factor (VEGF), CRP, neutrophil elastase 2 (ELA-2), lactoferrin, neutrophil gelatinase-associated lipocalin (NGAL), resistin, thrombospondin, and bactericidal/permeability-increasing protein (BPI). The performance of these biomarkers was evaluated over 95 samples of synovial fluid and the MSIS criteria were used to classify 29 PJIs and 66 aseptic joints. All patients were being evaluated for a revision hip or knee arthroplasty, including patients with systemic inflammatory disease (11 patients, of whom four were taking immune system modulating medications) and those already receiving antibiotic treatment. Out of the 16, five biomarkers demonstrated 100% sensitivity and specificity for the diagnosis of PJI: human a-defensin 1–3, neutrophil elastase 2, bactericidal/permeability-increasing protein, neutrophil gelatinase-associated lipocalin, and lactoferrin.[8,9,22] The most promising synovial fluid biomarker in terms of sensitivity and specificity for PJI seems to be AD.[15–23] Alpha defensin is an antimicrobial peptide that is secreted by human neutrophils in response to pathogenic presence.[36] It then integrates into the pathogen's cell membrane and causes rapid killing of the pathogen, thus providing antimicrobial support to the immune system.[37] Alpha defensin can be detected by the laboratory-based alpha defensin enzyme-linked immunosorbent assay (ELISA) or using an alpha defensin test kit.

## Alpha defensin immune assay

The ELISA test has demonstrated the highest ever reported accuracy for PJI diagnosis, but has to be performed in a laboratory and requires more time for response compared to the quicker lateral flow test. Bingham et al[17] obtained 100% sensitivity and 95% specificity of AD-1 assay in 57 patients and compared AD-1 assay with other clinical tests (cell count, culture, erythrocyte sedimentation rate, and C-reactive protein), showing that AD-1 assay results outperformed the other tests but did not reach statistical significance except for the sensitivity of the erythrocyte sedimentation rate. Deirmengian et al[18] compared the sensitivity and specificity of the synovial fluid AD immunoassay and LE in 46 patients, 23 with aseptic prosthesis loosening and 23 matching the MSIS criteria for PJI. AD correctly diagnosed 100% of PJI, whereas LE was able to correctly identify 78% of PJI. The assay for AD was optimized to operate at a cut-off value of 5.2 mg/L (lower limit of detection 1.56 mg/L) and the average AD concentration among infected samples was 59.6 mg/L, more than 30 times greater than the average concentration found in the aseptic samples (1.92 mg/L). In 18 out of 23 aseptic samples AD was totally undetectable.

Wyatt et al[19] in a systematic review and meta-analysis demonstrated a very high pooled diagnostic sensitivity and specificity of alpha defensin (sensitivity of 100% and specificity of 96%), remarkably better than those of the leukocyte esterase test. Li et al[21] conducted another systematic review that confirmed these results. On the other hand, they reported that strip tests are influenced by the quality of samples (the leukocyte esterase test as well as the Synovasure AD). Bonanzinga et al[22] checked the reliability of AD immunoassay in a prospective study, showing a sensitivity and specificity of 97%. The positive predictive value was 88%, and the negative predictive value was 99%. There were four false-positive patients, two presenting with metallosis and one with polyethylene wear. The false-negative case had a draining sinus, and intraoperative cultures were also negative.

## Alpha defensin lateral flow test

Lateral flow devices are a handy alternative that enable the detection of alpha defensin in synovial fluid 'in situ', even intraoperatively, and response is available in just ten minutes, making them markedly quicker than the ELISA test. Gehrke and colleagues[23] demonstrated the high accuracy of a new rapid alpha defensin lateral flow device (Synovasure AD test) on 195 joint aspirations comparing it to

the gold standard (MSIS criteria) for diagnosing periprosthetic joint infection: the results showed an overall sensitivity of 92.1% and a specificity of 100%. The positive predictive value was 100% (no false-positive values observed) and the negative predictive value was 95.2% (six false-negative cases). The overall accuracy was 96.9%, 189 of 195 cases.

However, the alpha defensin quick on-table lateral flow test (Synovasure) is not as accurate as the laboratory-based immunoassay,[24–25] but its high specificity combined with the advantage of a quick response time can make it useful for ruling in infection perioperatively.[26] Renz et al recently concluded that the AD lateral flow test for its statistical performance should not be used for screening, but rather as a confirmatory test for PJI.[27]

## Conclusion

Synovial biomarkers have shown encouraging results and they should be used as diagnostic adjuncts to synovial white cell count and culture bacteriology. This review confirms that the alpha-defensin assay has a role to play in the complex scenario of PJI diagnosis. The laboratory-based alpha defensin ELISA test demonstrated the highest ever reported accuracy for PJI diagnosis. The novel Synovasure alpha defensin test with a lateral flow device is an alternative format. Its main advantage is the availability of the results in ten minutes and its high specificity. Despite being slightly less accurate, it should be critically appreciated. This method could have its place in rapidly ruling in, and most importantly, ruling out a suspected PJI intraoperatively, ensuring better management and avoiding unnecessary treatments. However, every single test is associated with a high commercial price, which is a limiting factor. Its cost could be counterbalanced by shortening the hospital stay and diminishing the use of antibiotics, with a positive impact on bacterial resistance rates. Further cost-effectiveness studies will determine whether the costs of this new tool are justifiable.

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