

The Alpha Defensin-1 Biomarker Assay can be Used to Evaluate the Potentially Infected Total Joint Arthroplasty

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Abstract

Background Diagnosing a periprosthetic joint infection (PJI) requires a complex approach using various laboratory and clinical criteria. A novel approach to diagnosing these infections uses synovial fluid biomarkers. Alpha defensin-1 (AD-1) is one such synovial-fluid biomarker. However little is known about the performance of the AD-1 assay in the diagnosis of PJI.

Questions/purposes We sought to (1) determine the sensitivity and specificity of the AD-1 assay in a population of patients being evaluated for PJI, using the Musculoskeletal Infection Society (MSIS) criteria as the reference standard, and (2) compare the AD-1 assay with other currently

available clinical tests, specifically cell count, culture, erythrocyte sedimentation rate, and C-reactive protein.

Patients and Methods A retrospective review was performed of all patients undergoing workup for a PJI at our institution from January to June 2013. Sixty-one AD-1 assays were done in 57 patients. The group included 51 patients with 55 painful joints and six patients who underwent aspiration before second-stage reimplantation. Patients were considered to have a PJI if they met the MSIS criteria. We calculated the sensitivity and specificity of the AD-1 synovial fluid assay, and compared it with the sensitivity and specificity of the synovial fluid cell count, culture, erythrocyte sedimentation rate, and C-reactive protein. There were 19 diagnosed infections in the 61 aspirations, with 21 positive and 40 negative AD-1 assays. There were two false positive and no false negatives AD-1 assays.

Results The sensitivity and specificity for the AD-1 assay were 100% (95% CI, 79%–100%) and 95% (95% CI, 83%–99%), respectively. The sensitivity and specificity of the other tests ranged from 68% to 95% and 66% to 88%, respectively. The AD-1 assay results outperformed the other tests but did not reach statistical significance except for the sensitivity of the erythrocyte sedimentation rate.

Conclusion The sensitivity and specificity of the synovial fluid AD-1 assay exceeded the sensitivity and specificity of the other currently available clinical tests evaluated here but did not reach significance. The AD-1 assay offers another test with high sensitivity and specificity for diagnosing a PJI especially in the case where the diagnosis of PJI is uncertain, but larger studies will be needed to determine significance and cost effectiveness.

Level of Evidence Level III, diagnostic study. See the Instructions for Authors for a complete description of levels of evidence.

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Introduction

In many patients the diagnosis of periprosthetic joint infection (PJI) is not immediately obvious. The symptoms of PJI often are nonspecific, therefore making the diagnosis of PJI can be challenging [4]. Individually, currently available laboratory tests used to detect infection have been shown to be inadequate in diagnosing a PJI [5, 6, 10]. Even the definition of what constitutes a PJI is a topic of controversy [9]. To address inconsistencies in diagnosing a PJI with these tests, the Musculoskeletal Infection Society (MSIS) published a consensus statement providing a concise definition of a PJI [10]. The diagnosis of a PJI according to the MSIS definition requires either one of two major criteria (sinus tract communication with a prosthesis or a pathogen isolated by culture from two separate fluid samples), or four of six minor criteria (elevated erythrocyte sedimentation rate, elevated C-reactive protein, elevated white blood cell count, elevated percentage of polymorphonuclear neutrophils, presence of purulence, and greater than five neutrophils per high-power field on frozen section) [10]. Although clinically useful, this definition remains complex and time consuming.

The ideal method of diagnosis would be a single test or panel that is highly sensitive, specific, and simple to interpret. Synovial fluid biomarkers such as cytokines, inflammatory proteins, and antimicrobial peptides may provide such a tool, but the best biomarker or combination of biomarkers has yet to be determined [2]. One biomarker, the alpha defensin-1 (AD-1) protein has shown promise [7]. However to this point there has been no independent validation of the AD-1 assay in diagnosing a PJI.

We therefore sought (1) to determine the sensitivity and specificity of the AD-1 assay in an independent population of patients being evaluated for PJI, using the MSIS criteria as the reference standard, and (2) to compare the AD-1 assay with other currently available clinical tests, specifically cell count, culture, erythrocyte sedimentation rate, and C-reactive protein.

Patients and Methods

From January to June 2013 we sent fluid for AD-1 assay from patients undergoing workups for PJI. All patients who presented with failed or painful joint arthroplasty underwent infectious workup. Our standard infectious workup included C-reactive protein, erythrocyte sedimentation rate, white blood cell count, and joint aspiration for cell count, percentage of polymorphonuclear neutrophils, and cultures. All patients who had sufficient data to diagnose infection, based on the MSIS criteria, described below,

were included in the study. Sixty-one aspirations were done in 57 patients during a 6-month period. Patients who had hip and knee arthroplasties were included. Patients with autoimmune diseases were excluded from the study. Patients were subcategorized as having either painful joints or as before second-stage reimplantation of a previously infected arthroplasty. There were 51 patients with 55 painful joints and six patients who underwent aspiration before second-stage reimplantation for infection. Test kits were provided to our institution by the manufacturer (Synovasure[®]; CD Diagnostics Inc, Wynnewood, PA, USA) at no charge. The synovial fluid samples were sent to their certified laboratory (Citrano Medical Laboratories, Baltimore, MD, USA) for evaluation. The results of the AD-1 were reported as a qualitative yes or no result based on their predetermined quantitative cutoff of 7720 ng/mL. We were not informed of these quantitative results. Results were communicated to us the following day via email.

The results of our standard PJI workup then were collected and used to determine the presence of a PJI. Patients were considered to have a PJI if they met at least one of the major MSIS criteria or at least four of six minor criteria (or three of five if frozen sections were not performed). The sensitivity and specificity of the AD-1 assay were determined and 95% CIs were calculated [8]. The predetermined cutoffs for the other tests were greater than 1700 cells/mm³ for cell count, greater than 30 mm/hour for erythrocyte sedimentation rate, and greater than 10 mg/L for C-reactive protein [1, 5, 6, 11]. The sensitivity and specificity and the confidence intervals of the AD-1 assay were compared with the cultures, cell count, erythrocyte sedimentation rate, and C-reactive protein. Statistical analysis was performed with a McNemar's chi-square test to determine significant differences between studies.

Results

The AD-1 assay correctly diagnosed all 19 PJIs, with an overall sensitivity of 100% (95% CI, 79%–100%) and specificity of 95% (95% CI, 83%–99%). The sensitivity and specificity of the AD-1 assay were greater than those of the other tests (cultures, cell count, erythrocyte sedimentation rate, and C-reactive protein) we evaluated (Table 1). The sensitivity and specificity of the culture results alone were 69% (95% CI, 43%–86%) and 88% (95% CI, 72%–95%), respectively. Using a cell count cutoff greater than 1700 cells/mm³, we found a sensitivity of 95% (95% CI, 72%–99%) and specificity of 85% (95% CI, 69%–94%). Additionally, cutoffs of 10 mg/L for C-reactive protein and 30 mm/hour for erythrocyte sedimentation rate resulted in sensitivities of 79% (95% CI, 54%–93%) and 50% (95% CI, 29%–75%), and specificities of 66% (95% CI, 48%–80%)

Table 1. Sensitivity and specificity results

Results	Alpha defensin-1 assay	Culture	Cell count	Erythrocyte sedimentation rate	C-reactive protein
Sensitivity	100% (95% CI, 79%–100%)	69% (95% CI, 43%–86%) p = 0.065	95% (95% CI, 72%–99%) p = 0.239	50% (95% CI, 29%–75%) p = 0.013	79% (95% CI, 54%–93%) p = 0.067
Specificity	95% (95% CI, 83%–99%)	88% (95% CI, 72%–95%) p = 0.185	85% (95% CI, 69%–94%) p = 0.112	80% (95% CI, 63%–91%) p = 0.077	66% (95% CI, 48%–80%) p = 0.065

P value is for comparison with the AD-1 assay.

and 80% (95% CI, 63%–91%) respectively. When these tests were compared with the AD-1 assay only the sensitivity of the erythrocyte sedimentation rate reached statistical significance ($p = 0.013$). The remaining tests were not statistically significantly different from the AD-1 assay with the numbers available ($p > 0.05$).

Discussion

Diagnosing a PJI remains a clinical challenge, and although the definition was controversial, there seems to be emerging consensus that the MSIS criteria are reasonable for evaluating new diagnostic tools [9, 10]. However even with good definitions, we lack good tools to provide us with an unambiguous diagnosis of PJI. Synovial biomarkers may provide such a test. A preliminary study showed that the AD-1 assay can quantitatively detect the level of the AD-1 biomarker in synovial fluid and may prove to be highly sensitive and specific for diagnosis of PJIs [2]. However no independent study has confirmed the use of the AD-1 assay in diagnosing a PJI. We therefore sought (1) to determine the sensitivity and specificity of the AD-1 assay in a population of patients being evaluated for PJI using the MSIS criteria as the reference standard, and (2) to compare the AD-1 assay with other currently available clinical tests, specifically cell count, culture, erythrocyte sedimentation rate, and C-reactive protein.

Our study has some limitations. Owing to the size of our study we were unable to determine if the AD-1 assay was significantly better than the other tests evaluated, and followup at this time is less than 2 years. Additionally the AD-1 assay results were reported to us qualitatively only as positive or negative, and the quantitative results were not available to us for additional interpretation. Although the MSIS criteria are considered a good reference standard for infection and what we used to determine a PJI, the criteria are not a perfect measure for a PJI [10]. In addition, as a retrospective study, it is likely that selection bias may have influenced the results as there was no universally adhered-to approach that drove the decision to initiate a workup for

PJI or universal criteria that triggered a joint aspiration. Even so, in general, all patients who presented with a painful or failed joint arthroplasty underwent the same infectious workup including joint aspiration. In addition, although the results of the AD-1 assay were not used for clinical decisions, they were known to the treating surgeon which may have introduced bias.

The results of the AD-1 assay had excellent sensitivity and specificity and were consistent with previously reported data [3]. The current study is the first independent study, to our knowledge, to confirm that the AD-1 can be used for workup and diagnosis of a PJI. The AD-1 assay correctly identified all 19 PJIs, indicating that the AD-1 assay not only could be used in addition to other clinically available tests, but also may be a good screening tool for PJIs.

The results from our other diagnostic tests are consistent with previously reported data which also suggests that our data set is reliable [11]. When compared with the other tests we evaluated the AD-1 assay was at least equivalent, with the numbers available. However, a larger study is needed to determine if the AD-1 assay has a significantly better sensitivity and/or specificity owing to our relatively small study size.

We did have two false positive AD-1 assays. The first false positive was for a patient with an elevated cell count of 7800 cells/mm³, but normal C-reactive protein, erythrocyte sedimentation rate, percentage of polymorphonuclear neutrophils, and negative culture. The report noted that the fluid was hemolyzed and that caution should be used in the interpretation of the result. This patient subsequently underwent a revision hip arthroplasty for aseptic loosening secondary to osteolysis. Subsequent infectious workup since revision has been negative. The second false positive was in a patient with a known knee infection before second-stage reimplantation. This patient had an elevated C-reactive protein of 23 mg/L, erythrocyte sedimentation rate of 35 mm/hour, and cell count of 9300 cells/mm³, but negative culture and normal percentage of polymorphonuclear neutrophils. The patient was determined to be clear of infection and underwent second-stage reimplantation, and now is

asymptomatic but has not had any repeat infectious workup since the last procedure. Currently both patients with false positive results are doing well with no signs of infection. Both patients are greater than 1 year after surgery, but will need continued close followup as there still could be a possibility that these are unrecognized infections and not false positive results.

The sensitivities and specificities of the cultures, cell count, erythrocyte sedimentation rate, and C-reactive protein were consistent with historically reported values [11]. The AD-1 assay exceeded all of these individual tests in diagnosing and ruling out a PJI with a higher sensitivity and specificity, but did not reach statistical significance except in the case of the erythrocyte sedimentation rate. Additionally, the synovial samples with false positive AD-1 assays had a false positive erythrocyte sedimentation rate, C-reactive protein, cell count, or some combination of multiple false positives, suggesting some underlying inflammation which also may have elevated the AD-1 biomarkers. This suggests that as with other studies, AD-1 can be falsely elevated secondary to a noninfectious inflammation process, although likely to a lesser degree than erythrocyte sedimentation rate, C-reactive protein, and cell count.

Although we had a small group of patients undergoing testing with a novel assay tool, we showed that synovial biomarkers may prove to be useful in diagnosing PJIs compared with other markers of inflammation such as erythrocyte sedimentation rate, C-reactive protein, and cell count, and may provide one test for diagnosis of a PJI with high sensitivity and specificity. The high sensitivity of the AD-1 assay may make it a useful and effective screening tool to rule out a PJI. However larger numbers need to be obtained to determine a significant difference between the AD-1 assay and the other clinical tests evaluated.

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References

1. Costa CR, Johnson AJ, Naziri Q, Maralunda GA, Delanois RE, Mont MA. Efficacy of erythrocyte sedimentation rate and C-reactive protein level in determining periprosthetic hip infections. *Am J Orthop (Belle Mead NJ)*. 2012;41:160–165.
2. Deirmengian C, Hallab N, Tarabishy A, Della Valle C, Jacobs JJ, Lonner J, Booth RE Jr. Synovial fluid biomarkers for periprosthetic infection. *Clin Orthop Relat Res*. 2010;468:2017–2023.
3. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Booth RE Jr, Parvizi J. The alpha-defensin test for periprosthetic joint infection outperforms the leukocyte esterase test strip. *Clin Orthop Relat Res*. 2014 June 19. [Epub ahead of print].
4. Del Pozo JL, Patel R. Clinical practice: infection associated with prosthetic joints. *N Engl J Med*. 2009;361:787–794.
5. Dinneen A, Guyot A, Clements J, Bradley N. Synovial fluid white cell and differential count in the diagnosis or exclusion of prosthetic joint infection. *Bone Joint J*. 2013;95:554–557.
6. Ghanem E, Parvizi J, Burnett RS, Sharkey PF, Keshavarzi N, Aggarwal A, Barrack RL. Cell count and differential of aspirated fluid in the diagnosis of infection at the site of total knee arthroplasty. *J Bone Joint Surg Am*. 2008;90:1637–1643.
7. Jacovides CL, Parvizi J, Adeli B, Jung KA. Molecular markers for diagnosis of periprosthetic joint infection. *J Arthroplasty*. 2011;26(6 suppl):99–103.e1.
8. Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med*. 1998;17:857–872.
9. Parvizi J, Jacovides C, Zmistowski B, Jung KA. Definition of periprosthetic joint infection: is there a consensus? *Clin Orthop Relat Res*. 2011;469:3022–3030.
10. Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ, Garvin KL, Mont MA, Wongworawat MD, Zalavras CG. New definition for periprosthetic joint infection: from the Workgroup of the Musculoskeletal Infection Society. *Clin Orthop Relat Res*. 2011;469:2992–2994.
11. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. *J Bone Joint Surg Am*. 1999;81:672–683.