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Abstract:

Background:

Synovial biomarkers have recently been adopted as diagnostic tools for prosthetic joint infection (PJI) but their utility is uncertain. The purpose of this systematic review and meta-analysis was to synthesise the evidence on the accuracy of alpha-defensin immune-assay and leucocyte esterase colorimetric test strip for the diagnosis of PJI, compared to the Musculoskeletal Infection Society diagnostic criteria.

Methods:

We performed a systematic review to identify diagnostic technique studies evaluating the accuracy of alpha-defensin or leukocyte esterase in the diagnosis of PJI. OVID MEDLINE, EMBASE, ACM, ADS, arXiv, CERN DS, Crossref DOI, DBLP, Espacenet, Google Scholar, Gutenberg, Highwire, IEEExplore, Inspire, JSTOR, OAlster, Open Content, Pubget, PubMed and Web of Science entries for the last 10 years were searched along with grey literature. Classification of studies and data extraction were performed independently by two reviewers. Data extraction permitted meta-analysis of sensitivity, specificity with construction of receiver operator characteristic curves for each test.

Results:

We included 11 eligible studies. The pooled diagnostic sensitivity and specificity of alpha-defensin (six studies) for PJI were 1.00 (95% CI 0.82-1.00) and 0.96 (95% CI 0.89-0.99) respectively. The Area Under the Curve (AUC) for alpha-defensin and PJI was 0.99 (95% CI 0.98-1.00). The pooled diagnostic sensitivity and specificity of leucocyte esterase (five studies) for PJI were 0.81 (95% CI 0.49-0.95) and 0.97 (95% CI 0.82-0.99) respectively. The AUC for leucocyte esterase and PJI was 0.97 (95% CI 0.95-0.98) There was substantial heterogeneity between studies for both diagnostic tests.

Conclusions:

The diagnostic accuracy for PJI was high for both tests. Given the limited number of studies, more independent research on these tests is warranted given the large cost difference between the tests.

Level of Evidence:

Level 2 Testing of previously developed diagnostic criteria with consistently applied reference standard

Introduction:

Periprosthetic joint infection (PJI) is a rare complication affecting between 0.7 and 2.4% of patients receiving joint arthroplasty¹⁻⁹. PJI after hip and knee arthroplasty in particular has extremely negative effects on physical, emotional, social and economic aspects of a patient's life¹⁰. Early diagnosis can lead to less radical treatment with debridement and retention of prostheses instead of one or two-stage revision.

Establishing a diagnosis of infection promptly¹¹ is therefore of paramount importance, yet may be very challenging as the classic clinical features may be absent and a painful joint replacement may be caused by other pathologies. The Musculoskeletal Infection Society (MSIS) has developed diagnostic criteria to standardize and facilitate this diagnostic process (Table 1)¹². The search for a single diagnostic test with the requisite accuracy, sensitivity and specificity has yielded numerous biomarkers as potential candidates – the term biomarker meaning a biologically pertinent molecule that can be evaluated objectively to indicate the presence of a disease or biological state. Alpha-defensin¹³ and leucocyte esterase¹⁴ are currently among the most promising.

Alpha-defensin is an antimicrobial peptide that is released naturally from activated neutrophils. The peptide then integrates into, and destroys, the pathogens cell membrane¹⁵. The alpha-defensin immunoassay was developed from both genomic and proteomic studies and provides a qualitative result specific for synovial fluid. The advantages of this test include its simplicity and standardization whilst a disadvantage is its relatively high cost of £500 per test (\$760 US) (Synovasure, Zimmer).

Leucocyte esterase is an enzyme secreted by activated neutrophils recruited to areas of infection. Detection of leucocyte esterase has been used for many years in urinalysis to diagnose urinary infection¹⁶. The leucocyte esterase colorimetric strip test is performed by applying fluid to a reagent test strip. A detergent on the strip lyses the neutrophils within the fluid and this releases esterase which catalyses a reaction leading to formation of a violet dye. Advantages of this test are that it is quick, easy and inexpensive at 11 pence (17 cents US) per test (Combur-7, Roche). A potential disadvantage is the invalidation of the result by blood contamination¹⁷ although this has been addressed by centrifugation prior to application of the fluid¹⁸. Both of the tests describe require a synovial fluid sample.

The purpose of this systematic review and meta-analysis was to synthesise the available evidence on the accuracy of alpha-defensin and leucocyte esterase in the diagnosis of PJI.

Materials and Methods:

We used a rigorous and systematic approach conforming to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines¹⁹ (Appendix) and the critical evaluation of studies relating to diagnosis of PJI²⁰.

Protocol

A protocol was registered online with PROSPERO (CRD42015023704) before commencing the review as recommended by PRISMA.

Search strategy

We searched all studies indexed in MEDLINE and Embase on the Ovid platform, ACM, ADS, arXiv, CERN DS, Crossref DOI, DBLP, Espacenet, Google Scholar, Gutenberg, Highwire, IEEEExplore, Inspire, JSTOR, OAlster, Open Content, Pubget, PubMed and Web of Science from 1st June 2005 until 30th May 2015 years using the search strategy shown as applied in MEDLINE and Embase in Table 2. We also evaluated the grey literature with hand searches of six major Orthopaedic journals over the last five years. The bibliographies of the relevant articles were then cross-checked for articles not identified in the search. Studies in patients of all age groups were included. No language restrictions were applied, which is an important consideration with the perceived international interest in treatment of infected hip prostheses. The screening of studies was performed by two independent assessors with any disagreements resolved by a third reviewer. An Excel spreadsheet was constructed to summarise the findings of relevant studies.

Eligibility criteria

We included all diagnostic studies that enrolled patients with true diagnostic uncertainty in the setting of PJI. Tests of interest were alpha-defensin assay and leucocyte esterase test scoring ++. Eligible studies had a reference standard for diagnosing prosthetic joint infection using the MSIS diagnostic criteria.

Screening

A total of 1,796 records were identified from searching the literature. The titles and abstracts were screened to identify potentially useful articles for inclusion in this systematic review. After screening, 30 full articles were assessed in detail for

eligibility against criteria. A PRISMA flow diagram of the progression of studies through this systematic review is provided in Figure 2.

Data extraction

Two of the authors (MCW and SKK) worked independently to extract the data using standardized forms. We extracted data on sensitivity, specificity, likelihood ratio's, participants, joint involved, diagnostic test performed, cut off or range definitions of the tests, whether the cut-offs were derived with the use of receiver operator characteristic curves or predetermined by the study authors plus the nature and characteristics of the reference standard test. Quality assessment of each study was also performed using the QUADAS-2 tool²¹.

Statistical analyses

Overall sensitivity and specificity values for the diagnosis of PJI were pooled using the bivariate meta-analysis framework²². The bivariate model is an improvement and extension of the traditional summary receiver operating characteristic (sROC) and jointly models sensitivity and specificity as the starting point of the analysis and hence may be more reliable for estimating diagnostic accuracy^{23, 24}. The sROC curve shows the consistency of results across studies and therefore whether there was a uniform Receiver Operator Curve (ROC) curve over all studies. The sROC curve data-points come from regression analysis of each study whilst the ROC curve data-points come from each threshold. In addition the area under the curve (AUC) depicts the accuracy of the test. The bivariate model employs a random effects approach, which takes into account the heterogeneity beyond chance between studies. In addition, it also accounts for between-study correlation between underlying sensitivity and specificity, caused by the use of different thresholds across studies. I^2 was used to assess inconsistency between studies. An I^2 statistic is the proportion of variability across studies due to patient population variability rather than to sampling error. I^2 lies between 0% and 100%. A value of 0% indicates no observed heterogeneity, and values greater than 50% may be considered substantial heterogeneity *A priori* hypotheses to explain potential heterogeneity included site of the prosthesis and diagnosis of infection occurring at different time points.

Pooled positive and negative likelihood ratios were calculated using the summary estimates of sensitivity and specificity. Potential sources of heterogeneity across

studies could not be investigated because of the limited number of studies. In addition, tests for publication bias (e.g. Egger's test) require at least 10 studies and lower heterogeneity to be useful and valid, therefore we were unable to investigate for publication bias. All analyses were conducted using Stata version 14 (Stata Corp, College Station, Texas) and the `–midas–` and `–metandi–` commands were used for all analyses.

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Results:

There were 10 articles reporting 11 evaluations^{13, 14, 17, 18, 25-30} contributing to our estimates of diagnostic accuracy for both tests. Six studies explored the diagnostic accuracy of alpha-defensin for PJI^{13, 17, 25-27, 30}, while the remaining five studies explored the diagnostic accuracy of leucocyte esterase for PJI^{14, 17, 18, 28, 29}. Study characteristics are summarised in Table 3. All of the included studies were published within the last five years and originated from the USA. The largest contribution for the alpha-defensin test was from Deirmengian and colleagues^{13, 17, 26, 30} but there was no overlap in patients within these studies. The total number of patients contributing to the meta-analyses of alpha-defensin and leucocyte esterase were 2,321 and 545 respectively and involved 613 PJIs.

Quality assessment

In Table 4 the QUADAS-2 assessments for each study are reported. Using the QUADAS-2 tool those studies identified had a mean score of 13.3 (range 12 to 14) where a maximum score is 14. This indicates that the studies used in this meta-analysis were of good quality.

Diagnostic value of alpha-defensin for prosthetic joint infection

The pooled diagnostic sensitivity and specificity of alpha-defensin for PJI were 1.00 (95% CI: 0.82 to 1.00) and 0.96 (95% CI: 0.89 to 0.99) respectively (Figure 2). There was substantial heterogeneity between studies; I^2 (95% CIs) for sensitivity and specificity values were 98.2 (95% CI: 97.5 to 98.9) and 98.8 (95% CI: 98.4 to 99.2) respectively. The pooled Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), and diagnostic score were 27.0 (95% CI: 9.0 to 80.6), 0.00 (95% CI: 0.00 to 0.22), and 8.94 (95% CI: 4.73 to 13.15) respectively. The AUC for alpha-defensin and PJI was 0.99 (95% CI: 0.98 to 1.00) (Figure 3).

Diagnostic value of leucocyte esterase for prosthetic joint infection

The pooled diagnostic sensitivity and specificity of leucocyte esterase for PJI were 0.81 (95% CI: 0.49 to 0.95) and 0.97 (95% CI: 0.82 to 0.99) respectively (Figure 4). There was substantial heterogeneity between studies, I^2 (95% CIs) for sensitivity and specificity values were 94.6 (95% CI: 91.4 to 97.9) and 93.3 (95% CI: 89.0 to 97.6) respectively. The pooled PLR, NLR, and diagnostic score were 23.9 (95% CI: 3.8 to

152.1), 0.19 (95% CI: 0.06 to 0.66), and 4.82 (95% CI: 2.27 to 7.36) respectively. The AUC for leucocyte esterase and PJI was 0.97 (95% CI: 0.95 to 0.98) (Figure 5).

Discussion:

The prompt diagnosis of PJI remains a clinical challenge due to the diverse clinical presentations of patients suffering from this complication and the overlap of some of these features with other diagnoses and causes of failure of joint arthroplasty. The distinction between PJI and other causes of failure is important as the surgical management and chance of a successful outcome differs according to the mode of failure and thus the treatment strategy employed.

This systematic review has shown that alpha-defensin is extremely sensitive and specific in identifying PJI and that leucocyte esterase is slightly less sensitive, but also extremely specific. Both are therefore good candidates for diagnostic biomarkers. Traditional tests to diagnose PJI are less effective. In a study examining diagnostic accuracy for inflammatory serological markers in PJI³¹ the pooled sensitivity and specificity for ESR were 88% (95% CI: 86% to 90%) and 70% (95% CI: 68% to 72%) respectively. The pooled sensitivity and specificity for CRP were 97% (95% CI: 93% to 99%) and specificity 91% (95% CI: 87% to 94%). However this study did not use the MSIS criteria as its gold standard.

The strengths and potential limitations of this analysis deserve mention. Our meta-analysis is the first of its kind and it examined nearly two thousand patients in studies that were performed to a very high standard. All studies included in the review used the MSIS criteria, thereby minimising classification bias by using a common and widely accepted reference standard¹². The studies in our meta-analysis examined a large spectrum of joint arthroplasty including shoulder, hip and knee replacements, suggesting that the findings of our study can be generalised to several clinical scenarios. A number of the studies on alpha-defensin came from the same research group, which is unsurprising given the relative novelty of the test. This could hamper the generalisation of our findings; however, these findings were replicated by other groups. The limited number of studies precluded the ability to explore for publication bias and potential sources of heterogeneity. None of the studies reported on blinding which potentially may have introduced selection bias. Further large-scale and rigorous studies are warranted to evaluate the use of these synovial markers as diagnostic tools for PJI.

Alpha-defensin is substantially more expensive (£500 GBP per test) than leucocyte esterase (£0.11 GBP per test), but more specific in diagnosing PJI, it may be that they both have a clinical role as biomarkers. We recommend that further comparisons are made between these two promising biomarkers and traditional diagnostic tests to assess their relative effectiveness and cost effectiveness.

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