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BRIEF REPORT

Measuring synovial fluid procalcitonin levels in distinguishing cases of septic arthritis, including prosthetic joints, from other causes of arthritis and aseptic loosening

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Abstract
Objectives Differentiating septic arthritis from non-septic arthritis can be challenging as the clinical pictures are similar and an efficacious diagnostic test is not yet available. Our objectives in this study were to establish if procalcitonin (PCT) could be reproducibly measured from synovial fluid, if there is a difference in synovial procalcitonin values between patients with septic and non-septic arthritis, respectively, including those with implants and to determine cut-off levels that could be used as a practical tool in the management of these conditions.
Methods Using a standard serum assay, synovial fluid PCT levels were measured retrospectively in 26 septic and 50 non-septic predefined arthritis cases. The reproducibility of synovial PCT was also assessed at various concentrations.
Results Synovial PCT can be measured and is reproducible. In this cohort, statistically significant higher synovial PCT levels were found in cases of septic arthritis than in non-septic arthritis. Sensitivities, specificities and positive and negative predictive values varied at different cut-off levels.
Conclusion The test could be added to other microbiological and biochemical tests and may be used to supplement other clinical, radiological and laboratory findings in the assessment of patients with acute painful joints. In our cohort, findings of very high synovial PCT levels supported an infection process, including in prosthesis-related infections. The high negative predictive value of low synovial PCT levels could exclude infection in both native and prosthetic joints. Larger prospective studies are needed to further validate these results and to examine the cost effectiveness of synovial PCT.

Keywords Procalcitonin · Synovial · Pyogenic · Septic arthritis · Prosthetic joint infection

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Background

Clinical features of septic (pyogenic or bacterial) arthritis, including prosthetic joint infection (PJI), can mimic those of non-septic arthritis (e.g. degenerative, crystal and other inflammatory arthritis). In addition, manifestations of aseptic loosening can also resemble those of PJI. Consequently, differentiating various forms of arthritis clinically can prove to be a dilemma for attending clinicians, and studies have shown that the cause of arthritis remains unknown in about 16–36 % of patients [1]. Joint aspiration for cytology examination and bacterial culture, in combination with clinical, radiological and biochemical findings, remain the standard tests for the diagnosis, but none of these have a satisfactory efficacy, sensitivity or specificity [1, 2]. Although a true bacteriological culture can be regarded as a "gold standard" diagnostic test, in reality this approach is time consuming, especially when these infections require urgent antimicrobial treatment; in addition, a negative bacteriological culture does not always exclude an infective process, especially in those who has been on empirical antimicrobials prior to sampling. PCR assays

have been used to detect causative bacterial agents, but sensitivities and specificities vary among the different assays and are not routinely available in many UK hospitals [3].

Strategic biomarkers are needed to assist in a rapid diagnosis and differentiation of these cases. The utility of serum procalcitonin (PCT) in detecting bacterial infection has been recognized in multiple studies, and different serum cut-off levels have been suggested for various clinical conditions [4–10]. PCT has been previously evaluated as a biomarker for differentiating septic from non-septic arthritis with conflicting results. The variability in these results may be explained by both the presence or absence of systemic inflammatory response syndrome, the assays used and/or the whole study design, with some of these studies excluding certain groups of patients making their results less representative of real-life situations [11–17]. At a cut-off value of ≥0.3 μg/L, however, serum PCT has been found to be very specific (98 %), although its sensitivity is <35 %, particularly in PJIs [14–17]. The objectives of this study were to establish if PCT measurement from synovial fluid (usually validated as a serum assay) is reproducible and to evaluate the usefulness of measuring PCT levels directly from synovial fluid for differentiating septic arthritis, including PJI, from other forms of arthritis and aseptic loosening. We have also produced Receiver Operating Characteristic (ROC) analysis and calculated the sensitivity, specificity and predictive values for various synovial fluid PCT cut-off levels in predefined cases of septic and non-septic arthritis, including where an implant is present. To our knowledge this is the first report presenting such data.

Methods

This non-interventional, unblinded comparative study was performed at the Royal Hampshire County Hospital (Hampshire Hospitals NHS Foundation Trust), Winchester, UK. The study received ethical approval from the Health Research Authority NRES committee South West, Exeter, UK REC No. 12/SW/0070.

Using a standard quantitative PCT enzyme immunoassay kit (Brahms Diagnostica, Berlin, Germany) on a mini VIDAS analyser (BioMérieux, Marcy l'Etoile, France), we measured synovial PCT level retrospectively in 76 adult patients. The patients were categorized into two groups, with those in group A (n = 26) diagnosed with septic arthritis (eight cases of prosthesis-related infections and 18 cases of native joint arthritis) and those in group B (n = 50) with non-septic arthritis (including 6 cases of aseptic prosthetic loosening). Case selection, definition, inclusion and allocation to each group were based on clinical, radiological, microbiological culture and biochemical results, the patients' final diagnosis, including intraoperative findings in implant-related cases and response to treatment. Patients without a diagnosis suitable for placement in either group A or B or patients who had conflicting clinical and diagnostic findings were excluded from the study, including those who possibly or probably had a clinical infection, but for whom no confirmatory positive bacteriological cultures were available.

Between 2009 and 2012, synovial samples were taken from these patients aseptically as part of their routine investigations by appropriate clinical staff at different hospital source points. On arrival in the laboratory, the samples were processed for routine diagnostic tests, and the surplus from each sample was divided into aliquots and frozen at −20 °C for subsequent use in the PCT investigations. At the time of synovial PCT testing, the frozen samples were thawed and tested neat within 2 h; however, highly viscous joint fluid samples were tested neat and also diluted 1:4 (100 μL of joint fluid sample with 300 μL of serum-free reagent; ref. 66581, BioMérieux).

Given that synovial fluid is not the usual medium for which the PCT assay was designed, the reproducibility of synovial PCT measurements was also assessed on synovial PCT samples containing lower (≤0.05 and 1 μg/L) and higher (4.6 μg/L) PCT concentrations using a previously validated formula [18]. Statistical analysis was performed using SPSS ver. 20 software (SPSS, Chicago, IL) to compare the mean synovial fluid PCT scores of the two groups. We also performed a receiver operating characteristic (ROC) analysis and determined the sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) for various synovial fluid PCT concentrations.

Results

A total of 76 patients (26 in group A and 50 in group B) were enrolled in the study (Table 1). There was a statistically significant difference in the mean synovial fluid PCT values between the two groups (Fig. 1). The ROC analysis and the sensitivity, specificity and PPV and NPV for various synovial PCT values are given in Fig. 2.

Within the batch of synovial PCT samples containing different concentrations of PCT, the coefficient of variation (CV) was 0, 4.1 and 3.3 % at cut -ff points of ≤0.05, 1, and 4.6 μg/L, respectively.

Discussion

Septic arthritis is potentially the most dangerous and destructive form of acute arthritis. Differentiating this from

Table 1 Age, male to female ratio and diagnosis in each study group

Patient characteristics	Group A[a]	Group B
Median age, years (range)	78.7 (43–88)	66.5 (30–90)
Diagnosis (number of cases)	Prosthetic joint infection (PJI) (8)[b]	Aseptic loosening (6)[b]
	Native joint septic arthritis (18)	Crystal arthropathy (20)
		Osteoarthritis, rheumatoid arthritis, psoriatic arthritis and other inflammatory and non specific arthritis (24)
Total number of cases (male:female)	26 (15:11)	50 (33:17)

[a] Microorganisms detected were *Staphylococcus aureus* (12 cases), other *Staphylococci* spp. (4 cases), β-haemolytic *Streptococci* (3e cases), *pneumococci* (1 case), *enterococci* (1 case) and *Escherichia coli*, *Proteus* spp. and other coliforms (remaining 5 cases)

[b] Mixture of knee and hip arthroplasties. Median synovial procalcitonin (PCT) level in the PJI group was 3.12 vs. 0.05 μg/L (aseptic loosening group). Only two cases of PJI had concomitant positive blood cultures. The highest synovial PCT levels were seen in *S. aureus* infections regardless of the presence of concomitant positive blood cultures. Note this is just an observation and the numbers are too small to extract statistical power

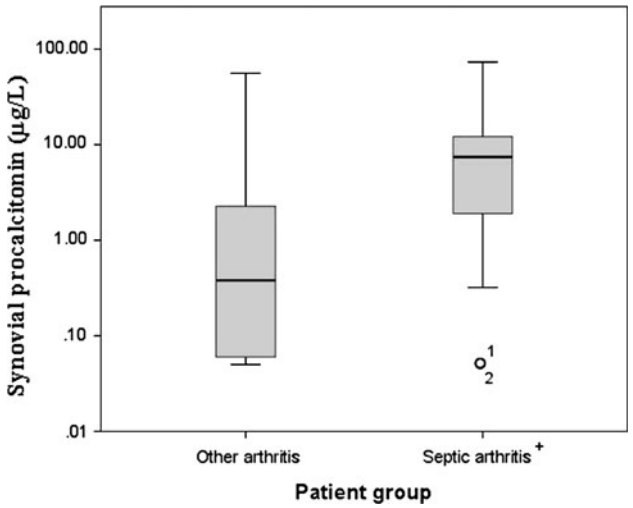

Fig. 1 Synovial concentration of procalcitonin (PCT) according to the diagnosis of septic vs. non-septic (other) arthritis. Under the assumption of no equal variance, the independent-samples *t* test suggested that there was a significant difference in the mean values for non-septic arthritis [in μg/L; median 0.415, range 0.05–56.06, mean 2.61; standard deviation (SD) 8.22] and septic arthritis (in μg/L; median 7.435, range 0.05–73.33, mean 10.37, SD 14.95; $p = 0.020$). *1*, *2* Patients with confirmed septic arthritis and low synovial PCT; both had been on appropriate antibiotics for some time prior to sampling although this treatment may not be the reason for the low PCT level. *Plus symbol* All cases had a positively significant microbiological culture in the synovial fluid; there was no apparent relation between the nature of the organism and the synovial PCT value. PCR was not used due to lack of availability in-house and funding. Blood cultures were taken in all these cases in this group (group A), with only 6 of the 26 cases with a concomitant (same isolate) positive blood culture

non-septic causes of arthritis can be a challenge in the clinical setting. This is not only related to the similarities in clinical presentation but also to the lack of an efficacious diagnostic test. A systematic review has shown that the cause of arthritis remains unknown in about 16–36 % of patients [1]. A delay in the treatment of septic arthritis could have devastating consequences, patients tend to be started on prolonged intravenous antibiotic therapy. Bacteriological culture, although regarded as the "gold standard", can take a long time and may give false negative results, especially if patients have been on antibiotics prior to sampling.

Our results suggest that synovial PCT may be a valuable biomarker in supporting clinicians in differentiating septic from non-septic arthritis as in our study cohort group A patients (diagnosed with septic arthritis) had significantly higher mean synovial PCT values than group B patients (diagnosed with non-septic arthritis) (Fig. 1). However, as with any diagnostic test, synovial fluid PCT values have to be interpreted in the context of the clinical setting. Based on our cohort a synovial fluid PCT cut-off value of <0.5 μg/L is likely to exclude the diagnosis of septic arthritis with a NPV of 0.90 (95 % confidence interval 0.73–0.97) but a poor PPV (Fig. 2). Two patients in group A had synovial PCT values of <0.5 μg/L despite having a genuine infection: one had a low grade PJI due to coagulase-negative staphylococci (CoNS), and the other had a native joint infection due to *Escherichia coli*; both patients received the appropriate antibiotics prior to synovial fluid aspiration (Fig. 1; Table 1). However, there were seven other patients in group A who received antibiotics prior to sampling, and their synovial PCT levels remained elevated. Therefore, prior antibiotic therapy in these two cases does not entirely explain the low synovial PCT values, although the low grade PJI infection with CoNS may be a factor. In contrast, higher synovial PCT levels among group B patients were observed in cases of crystal arthritis, particularly where there was a concomitant malignancy; two of the highest values in this group were found in patients with a history of renal cell carcinoma and carcinoid tumour. In the absence of preexisting inflammatory conditions or malignancy, a value of >0.5 μg/L may also be highly supportive for the diagnosis of an infection process, and

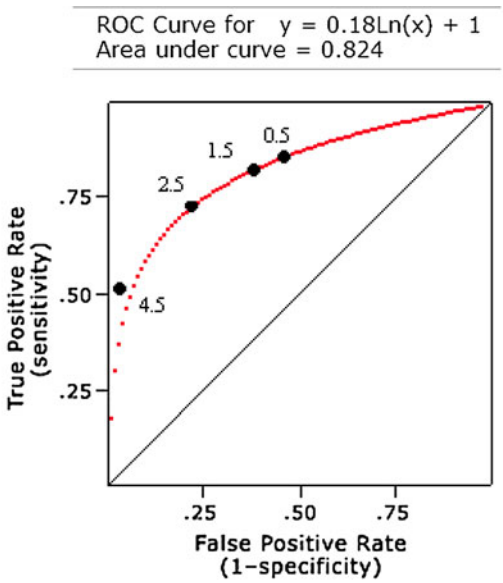

Fig. 2 Area under the receiver operating characteristic (*ROC*) curve and the ability of synovial PCT to distinguish septic arthritis from non-septic arthritis [sensitivity, specificity, positive and negative predictive values (95 % confidence interval)] at different cut-off levels

Cut-off µg/L	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)	PPV (95% Confidence Interval)	NPV (95% Confidence Interval)
0.5	0.88 (0.69-0.97)	0.57 (0.42-0.71)	0.52 (0.37-0.67)	0.90 (0.73-0.97)
1.5	0.85 (0.64-0.95)	0.62 (0.55-0.81)	0.59 (0.42-0.75)	0.89 (0.74-0.96)
2.5	0.69 (0.49-0.85)	0.78 (0.63-0.88)	0.62 (0.42-0.79)	0.83 (0.68-0.92)
4.5	0.54 (0.34-0.73)	0.94 (0.82-0.98)	0.82 (0.56-0.95)	0.79 (0.66-0.88)

overall synovial fluid PCT values of >4.5 µg/L provided a PPV 0.82 and NPV of 0.79 for the diagnosis of septic arthritis (Fig. 2). This cut-off value may be particularly valuable in cases of crystal arthritis with simultaneous septic arthritis, but further studies are needed to confirm this hypothesis.

Although previous reports have shown that serum PCT measurements lack sensitivity in the diagnosis of PJI, with some authors attributing this to the absence of a systemic inflammatory response in most PJI [14–17], our observations is that (apart from the one patient infected with CoNS discussed above) cases of PJI, regardless of the presence of concomitant positive blood cultures, were associated with higher synovial PCT values than cases of aseptic loosening (Table 1). We therefore believe that synovial PCT measurements warrant further evaluation in cases of early and delayed PJIs in larger centers and with appropriate controls.

From the limited number of tests that we have conducted, it appears that our PCT assay performs relatively well and is reproducible (CV range from 0 to 4.1 % at various cut-offs). The test takes up to 30 min to perform in the laboratory and may have the potential to become a point of care test performed by those who obtain the synovial fluid. Some of the limitations of this study include a relatively small number of patients and the absence of other biomarker assays performed concomitantly, including serum PCT, to compare to values reported in other studies and to synovial PCT values in this study. Additionally, the retrospective aspect of our study was a major limitation in terms of comparing synovial PCT values to full clinical details, criteria of systemic inflammatory response and the severity of arthritis. Nevertheless, our results provide some evidence that synovial fluid PCT may become a useful tool in the evaluation and management of patients with painful and swollen joints, including those with orthopaedic implants, and that it may supplement other clinical, radiological and laboratory findings in differentiating septic from other arthritis. Differentiating septic from non-septic arthritis with a simple biochemical test is a major challenge. Based on our findings, we believe that synovial PCT measurements would be more informative than serum PCT measurements in differentiating infection from non-infection cases of arthritis, particularly when the nature of the infection is more localized and in PJIs. Very high synovial fluid PCT levels (e.g. >4.5 µg/L) may support a diagnosis of an infective process with subsequent initiation or continuation of antibiotic therapy; at the same time, the high NPV of this measurement with lower synovial PCT levels (e.g. <0.5 µg/L) could aid clinicians to exclude a diagnosis of septic arthritis, directing them towards alternative diagnoses and prompting cessation of antibiotic therapy once started. This in turn could lead to a reduction in unnecessary antibiotic use, a valuable

aspect of antimicrobial stewardship in the era of rising antimicrobial resistance, as well as a change in the management of implant-related infections. Before synovial PCT becomes a routine diagnostic test, larger, prospective studies are needed to further validate these findings, define optimal synovial cut-off levels in both native and prosthetic joints and perform a cost-effectiveness analysis.

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Conflict of interest None.

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