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Alpha defensin, leukocyte esterase, C-reactive protein, and leukocyte count in synovial fluid for pre-operative diagnosis of periprosthetic infection

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Abstract

Synovial fluid analysis for diagnosis of prosthetic joint infections has gained increasing interest in the recent past when markers more specific for these infections than the serum ones have been identified. Despite the important steps forward, identification of a gold standard has not yet been identified. In this study, usefulness of alpha defensin, leukocyte esterase, C-reactive protein (CRP), and white blood cells (WBCs) in synovial fluids alone and in combination for diagnosis of prosthetic joint infection was evaluated. Synovial fluids from 32 infected and 34 not infected patients were analyzed. Sensitivity, specificity, positive and negative predictive values, diagnostic accuracy, and receiver-operating characteristic (ROC) curves were calculated for each parameter. Moreover, combination of coupled variables was also evaluated by logistic regression analysis. Sensitivity of alpha defensin, CRP, leukocyte count, and leukocyte esterase were 84.4%, 87.5%, 93.7%, and 93.8%, respectively. Specificity was 91.2% for leukocyte counts, 94.1% for alpha defensin, 97.0% for CRP, and 97.1% for leukocyte esterase. Diagnostic accuracy was 89.4% for alpha defensin, 92.4% for WBC counts and CRP, and 95.5% for leukocyte esterase. No statistical differences were observed in area under the curve (AUC) of the ROC curves of alpha defensin, CRP, and leukocyte counts. Logistic regression analysis applied to a model comprising all the variables showed an AUC higher than AUC of coupled variables. In conclusion, results of this study confirm the high sensitivity and specificity of synovial leukocyte esterase for diagnosis of prosthetic joint infection, also suggesting the need to assess a panel of markers to optimize diagnosis of these infections.

Keywords

α-defensin, leukocyte esterase, markers of infections, prosthetic joint infections, synovial fluid

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Introduction

Prosthetic joint infections (PJIs) represent the most devastating complication in prosthetic surgery requiring repeated surgeries, prolonged antibiotic therapy, and long rehabilitation periods. Consequently, PJIs heavily impact not only on patients' quality of life but also on the healthcare system because of the costs associated with their management.[1,2]

Because current surgical approaches to septic and aseptic prosthetic failure significantly differ,

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diagnosis of PJI is crucial for the correct patients' management. In the recent past, efforts have been made to identify specific and accurate markers of PJI in synovial fluid, where measurement of inflammatory parameters like leukocyte esterase, alpha defensin, and C-reactive protein (CRP) is characterized by higher specificity and sensitivity. In particular, leukocyte esterase has proven to be a reliable and rapid test for determination of diagnosis of PJI.[3–5] Moreover, a positive leukocyte esterase assay has been included in the diagnostic criteria of the International Consensus Meeting of Philadelphia as an alternative to synovial leukocyte count.[6]

White blood cells (WBCs) accumulate in synovial fluid of patients with infected prosthetic joints and are included among the minor criteria for definition of PJI.6 Nonetheless, WBC count is rather unspecific, since other inflammatory conditions such as rheumatoid arthritis and metallosis can lead to a high count. Moreover, cutoff values are still under debate, although a count higher than 10,000 cells/μL or 3000 cells/μL are generally considered suggestive for acute and chronic infections.[6]

The purpose of this study was to evaluate alpha defensin, leukocyte esterase, CRP, and leukocytes counts in synovial fluid of patients with suspected late infection of a prosthetic joint.

Methods

Patients' population and sample collection

A total of 66 outpatients referring to our Institute for suspected late PJI from October 2015 to March 2017 were considered. Inclusion criteria were a sufficient amount of synovial fluid for all determinations, and full clinical and laboratory data to allow for diagnosis of infection as detailed below. Exclusion criteria were insufficient amount of synovial fluid to perform all assays and samples for leukocyte counts excessively contaminated by blood. All patients were informed about the possibility that their samples could have been used for research studies and gave their consent. A total of 21 samples were obtained from the hip and the remaining 45 from the knee.

Synovial fluid was aseptically collected and distributed into serum tubes containing an acrylic gel and micronized silica (BD Vacutainer SSTTM, Becton Dickinson, Italy) for leukocyte esterase and CRP determination and into tubes not containing any additive for alpha defensin measurement. Moreover, another aliquot was used for synovial fluid culture. Diagnosis of PJI was made retrospectively using the definition of the International Consensus Meeting of Philadelphia,[6] but excluding elevated synovial fluid count or positive leukocyte esterase as a minor criterion. Therefore, a PJI was defined by the presence of a sinus tract communicating with the prosthesis or isolation of the same pathogen by culture from at least two separate tissue or fluid samples obtained from the affected prosthetic joints or having three of the following minor criteria: elevated erythrocyte sedimentation rate (ESR) and serum CRP concentration, high percentage of synovial fluid polymorphonuclear neutrophils, positive histology of periprosthetic tissue, and single positive culture.[6]

Alpha defensin determination

Alpha defensin concentrations in synovial fluid were measured by means of enzyme-linked immunosorbent assay (ELISA; Synovasure, CD Diagnostic, Wynnewood, Pennsylvania). Samples were centrifuged at 3000 r/min for 10 min to remove particulates and cellular debris which could interfere with the ELISA assay, which was performed within 6 h from sample collection. Alpha defensin determination was performed in accordance with manufacturer's instructions and results were read at 450 nm by means of a multiplate reader (MultiskanTM FC, Thermo Fisher Scientific, Carlsbad, CA, USA). The assay has been set up to operate at a cutoff value of 5.2 mg/L, providing results as a semiquantitative signal-to-cutoff ratio of 1.0.

Leukocyte esterase evaluation

Determination of leukocyte esterase in synovial fluid was performed by means of an enzymatic colorimetric strip containing different tests (Dirui Industrial Co Ltd, China). Samples were centrifuged at 3000 r/min for 10 min to limit interference caused by the presence of corpuscles. Then, one drop of the supernatant was placed on the specific pad of the strip, which was read after about 2 min. Production of purple color indicated the presence

of the enzyme. The strips were considered positive when graded +, ++, and +++ while traces or absence of color were considered negative.

Measurement of CRP

After sample centrifugation as described for leukocyte esterase test, synovial CRP was measured by an automated turbidimetric method using a specific reagent kit reacting goat anti-CRP antibody and patient CRP antigen in the sample into sedimentation complexes. The assay was run on ARCHITECT i2000 system (Abbott Laboratories, IL).

Leukocyte count in synovial fluid

Leukocyte count was performed on synovial fluid collected into K_3EDTA tubes by means of a cell counter (Sysmex XE 2100 Haematology Automated System, Sysmex Partec Italia, Italy) within 30 min from the arrival of the sample in the laboratory. Before analysis, samples were gently mixed for 10 s. Samples were analyzed in duplicate and the mean value calculated.

Microbiological cultures

Synovial fluid was centrifuged at 3000 r/min for 10 min at room temperature. The supernatant was discharged until a final volume of about 1 mL of liquid was reached and 100 µL of the resuspended pellet was plated on chocolate agar (CA), mannitol salt agar (MSA), MacConkey agar (MCA), Sabouraud agar (SAB) (all purchased from BioMerieux, Mercy L'Etoile, France), and inoculated into Brain Heart Infusion Broth (BHI, BioMerieux) and thioglycollate broth (TH, Thermo Fisher, Cornaredo, Milan, Italy). CA and MCA plates were incubated for 24 h in CO_2-enriched and ambient atmosphere, respectively; MSA and SAB plates were incubated aerobically for 48 h. BHI and TH broths were maintained for 15 days at 37°C and checked daily for bacterial growth.[7] When broths became turbid or at the end of the incubation period, aliquots of each broth were plated on CA and Schaedler (only TH broth) agar plates and incubated for 48–72 h in CO_2-enriched atmosphere and in anaerobiosis, respectively.

Microbial identification was performed on automated instrumentation Vitek 2 compact (bioMérieux).

Statistical analysis

Descriptive statistics were used to summarize laboratory values and patients' characteristics. Sensitivity and specificity, and positive and negative predictive values with 95% confidence intervals (CIs) were calculated. Diagnostic accuracy was also evaluated considering the following formula: (True positive + True negative)/(True positive + True negative + False positive + False negative). Since different cutoff values for CRP, leukocyte count, and leukocyte esterase have been proposed, we calculated sensitivity and specificity for two different cutoff values of each parameter. Cutoff values showing the highest diagnostic accuracy were used for further analysis. Receiver-operating characteristic (ROC) curves were calculated for alpha defensin, CRP, and WBC counts. The accuracy of combination of variables (alpha defensin with either leukocyte esterase or CRP or WBC counts, leukocyte esterase with either CRP or WBC counts and CRP with WBC count) was calculated by means of logistic regression analysis. A P value equal to or less than 0.05 was considered as statistically significant. All statistical calculations were performed on a free available tool for statistical computation (VassarStats: Website for Statistical Computation. Available at www.vassarstats.net) and on MEDCALC software (MEDCALC Statistical Software version 16.2.1; MEDCALC Software; Ostend, Belgium; 2016).

Results

A total of 66 patients were included in the analysis: 32 (Group A) were diagnosed as infected and 34 (Group B) as not infected (Table 1).

The two groups did not significantly differ for male/female ratio, age, and joint side (hip or knee). ESR and serum CRP were significantly higher in Group A than in Group B ($P < 0.001$).

Synovial fluid culture

Microbial growth was observed in 29 samples of Group A (90.6%) and in 2 samples of Group B (5.9%). Staphylococci were the predominant microorganisms isolated in synovial fluid samples: *Staphylococcus epidermidis, Staphylococcus aureus*, and other coagulase-negative staphylococci (*Staphylococcus lugdunensis, Staphylococcus capitis, Staphylococcus haemolyticus, Staphylococcus*

intermedius, and *Staphylococcus caprae*) were isolated in 6, 5, and 9 samples, respectively. Other Gram-positive cocci were *Enterococcus faecalis* (n=2), *Streptococcus agalactiae* (n=1), and *Streptococcus gordonii* (n=1).

Among Gram-negative bacilli, *Escherichia coli, Proteus mirabilis*, and *Morganella morganii* were isolated in one sample each, as well as *Propionibacterium acnes* and *Corynebacterium jeikeium*.

P. acnes and *Corynebacterium striatum* were isolated from synovial fluid culture of two patients in Group B, but, since none of the other criteria for PJI diagnosis was fulfilled, they were considered contaminants.

Synovial fluid analysis

Sensitivity, specificity, and positive and negative predictive values of synovial alpha defensin, leukocyte esterase, CRP, and WBC count are reported in Table 2. Mean signal-to-cutoff ratio of alpha defensin was 2.99 (95% confidence interval (CI): 2.37–3.61) in Group A and 0.35 (95% CI: 0.38–0.52) in Group B ($P<0.001$). Considering a signal-to-cutoff ratio of 1.0 as suggested by the manufacturer, 27/32 samples resulted positive in Group A and 32/34 negative in Group B, with a sensitivity of 84.4% and a specificity of 94.1%. Area under the ROC curve was 0.975 (95% CI: 0.903–0.998).

Synovial CRP levels were significantly higher in samples of Group A (mean: 34.1 mg/L, 95% CI: 27.1–41.1 mg/L) than in Group B (mean: 2.41 mg/L, 95% CI: 1.61–3.21 mg/L; $P<0.0001$). As shown in Table 2, a higher sensitivity was observed with a cutoff value of 7 mg/L than with a value of 10 mg/L, though maintaining the same specificity. Area under the ROC curve was 0.949 (95% CI: 0.865–0.988).

Mean synovial WBC were 22,740 cells/μL in Group A and 986 cells/μL in Group B ($P<0.0001$). Considering a cutoff value of 3000 cells/μL, sensitivity and specificity of synovial WBC count were 93.7% and 91.2%, respectively. By contrast, when the cutoff was set at 1600 cells/μL, sensitivity increased to 100% while specificity fell to 82.3%. Area under the ROC curve was 0.983 with 95% CI ranging from 0.915 to 0.995.

In Group A, leukocyte esterase was scored as 3+, 2+, and 1+ in 5, 15, and 10 patients, respectively, while in two cases a negative result was observed. In Group B, 30 samples resulted negative, in three samples leukocyte esterase was present in traces (a result considered negative), and a 1+ score was observed in one sample. Therefore, a sensitivity of 93.8% and a specificity of 97.1% was obtained with a cutoff value of 1+. A cutoff of 2+

Table 1. Patients' characteristics.

	Group A	Group B
Number of patients	32	34
Male/Female	18/14	19/15
Mean age (years)	69.7 (66.2–73.2)	66.5 (62.9–70.1)
Prosthetic joint		
Hip	10	11
Knee	22	23
Mean ESR (mm/h)	41.6* (35.7–47.5)	13.9 (10.6–17.2)
Mean serum CRP (mg/L)	36.0* (26.7–45.3)	2.33 (1.68–2.98)

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.
95% Confidence interval are reported in parenthesis.
*$P<0.001$ Group A versus Group B.

Table 2. Sensitivity, specificity, and positive and negative predictive values of synovial markers.

Cutoff	Alpha defensin	Leukocyte esterase		C-reactive protein		WBC Count	
	Ratio = 1.0	1+	2+	7.0 mg/L	10 mg/L	1600 cells/μL	3000 cells/μL
Sensitivity (%)	84.4 (66.5–94.1)	93.8 (77.8–98.9)	56.3 (37.9–56.2)	87.5 (70.1–95.9)	81.3 (62.9–92.1)	100 (86.6–100)	93.7 (77.8–98.9)
Specificity (%)	94.1 (78.9–98.9)	97.1 (82.9–99.8)	100 (87.3–100)	97.0 (82.9–99.8)	97.1 (82.9–99.8)	82.3 (64.8–92.6)	91.2 (75.2–97.7)
Positive predictive value (%)	93.1 (75.8–98.8)	96.8 (81.4–99.8)	100 (78.1–100)	96.5 (80.4–99.8)	96.3 (79.1–99.8)	84.2 (68.1–93.4)	90.9 (74.5–97.6)
Negative predictive values (%)	86.5 (70.4–94.9)	94.3 (79.5–99.0)	70.8 (55.7–82.6)	89.2 (73.6–96.5)	84.6 (68.8–93.6)	100 (84.9–100)	93.9 (78.4–98.9)

WBC: white blood cell.
95% confidence interval is reported in parenthesis.

Table 3. Logistic regression analysis.

	Area under the curve of pairwise combination		
	Leukocyte esterase	C-reactive protein	WBC Count
Alpha defensin	0.983 (0.915–0.999)	0.978 (0.907–0.999)	0.993 (0.932–1.00)
Leukocyte esterase	–	0.994 (0.935–1.00)	1.00 (0.946–1.00)
C-reactive protein	–	–	0.860 (0.753–0.933)

WBC: white blood cell.

led to an increase in specificity up to 100%, but sensitivity fell to 56.3%.

Diagnostic accuracy was 89.4% for alpha defensin; 90.9% and 92.4% for WBC counts with cutoff at 1600 and 3000 cells/μL, respectively; 89.4% and 92.4% for CRP with cutoff of 10 and 7 mg/L, respectively; and 95.5% for leukocyte esterase. No differences were observed between area under the curve (AUCs) of the ROC curves of alpha defensin, CRP, and WBC counts. Logistic regression analysis applied to a model comprising all the variables showed an AUC of 1.00 (95% CI: 0.946–1.00), which was higher or equal to the AUC of pairwise associated variables (Table 3).

Discussion

On the whole, our data evidenced the importance of synovial leukocyte esterase, alpha defensin, CRP, and leukocyte count for PJI diagnosis, being all these assays characterized by a diagnostic accuracy near or higher than 90% and an AUC for the ROC curves higher than 0.9. Sensitivity and specificity of leukocyte esterase, CRP, and WBC counts confirmed those previously reported in other studies,[3–5,8] while a lower sensitivity was observed for alpha defensin in respect to what has been published by other authors (84.3% vs 97%–100%).[9,10] False negative results for alpha defensin were observed when the infection was due to low virulent microorganisms such as coagulase-negative staphylococci and Corynebacteria, which may cause a limited inflammatory response with, consequently low levels of alpha defensin and, at a lesser extent, even of leukocyte esterase. Leukocyte esterase showed the highest diagnostic accuracy when a cutoff of 1+ was applied. Its low costs and easy use make it a valid option in the diagnostic workflow of PJI. Sensitivity of CRP increased notably when cutoff was reduced from 10 to 7.0 mg/L, though maintaining similar specificity. This finding underlines the importance to standardize CRP analysis since a wide variability in cutoff values has been reported by Sousa et al.[11] and Omar et al.[12]

Sensitivity of WBC count observed in this study was higher than that observed for alpha defensin and equal to that of leukocyte esterase. By contrast, the assay was characterized by the lowest specificity. A possible explanation is that the study included patients with concomitant inflammatory diseases such as metallosis or rheumatoid arthritis, which could affect WBC counts.

The limited population size and the distribution of infected and not infected patients which do not reflect the real prevalence of PJIs may have affected results of this study. The main reason is that because of the high costs of alpha defensin test, the assay was requested only for patients for whom there was a strong suspicion of infection. Moreover, our Institute is a referral center for bone and joint infection, and the prevalence of infected patients is higher than in other clinical settings. In conclusion, our results show that analysis of synovial fluid represents an important weapon in the diagnosis of PJI. The use of simple and time- and cost-saving assays, such as leukocyte esterase, CRP, and leukocyte count, may provide useful information to better define patients' management.

Declaration of conflicting interests

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