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ORIGINAL PAPER

Gram and acridine orange staining for diagnosis of septic arthritis in different patient populations

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Abstract
Purpose The sensitivity of Gram staining is known to be suboptimal for the diagnosis of native joint septic arthritis. We lack information about the accuracy of Gram compared to other microscopic staining techniques for predicting infection in different patient populations.
Methods This was a cohort study with cost evaluations at the Orthopaedic Service of Geneva University Hospitals (January 1996–October 2012).
Results Among 500 episodes of arthritis (196 with immunosuppression, 227 with underlying arthroplasties and 69 with gout or other crystals in synovial fluid), Gram staining revealed pathogens in 146 episodes (146/500, 29 %) or in 146 of the 400 culture-positive episodes (37 %). Correlation between the Gram and acridine staining of the same sample was good (Spearman 0.85). Overall, the sensitivity, specificity, positive predictive value and negative predictive value of Gram stain for rapid diagnosis of septic arthritis was 0.37, 0.99, 0.99 and 0.28, respectively, compared to microbiological cultures. Quite similar values were recorded across the different patient subpopulations, in particular for sensitivity values that were 0.33 for patients with prosthetic joint infections, 0.40 for immunosuppressed patients, 0.36 for patients under antibiotic administration and 0.52 for patients with concomitant crystalline disease.
Conclusions The sensitivity of Gram or acridine orange staining for a rapid diagnosis of episodes of septic arthritis is suboptimal compared to microbiological culture, regardless of underlying conditions, immunosuppression or antibiotic therapy. The sensitivity in the presence of synovial fluid crystals is moderate. Acridine orange and Gram stains are equivalent.

Keywords Septic arthritis · Gram stain · Acridine orange stain · Sensitivity · Specificity

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Introduction

A low concentration of microbial pathogens in the otherwise sterile synovial fluid is sufficient to trigger a severe inflammation that may lead to severe cartilaginous damage. In contrast to abscesses in soft tissue infections, this low bacterial concentration may remain undetected by microscopic examination. Therefore, medical and surgical teams frequently await the results of synovial fluid cultures for the definitive diagnosis of septic arthritis, in the presence of clinical signs and purulent synovial fluid in joint aspirates. As cartilage damage is believed to occur within a few days in most non-gonococcal arthritis cases, many surgeons rapidly drain and start antibiotic treatment before receiving the microbiological culture results [1, 2], unless there is a strong suspicion of recurrent gout or pyrophosphate-related joint inflammation [3, 4].

The sensitivity of Gram staining is known to be suboptimal for the diagnosis of native joint septic arthritis [1, 5–8]. Previous reports usually evaluated small sized groups including less than 80 patients [4–6, 8–13] and mainly focused on episodes of native joint septic arthritis [4–6, 8–14]. Few studies compared the efficacy of Gram with other staining procedures [15], e.g. acridine orange, or evaluated different patient populations, such as those with prosthetic joint infections [16, 17], various immunocompromised conditions or concomitant gout [8, 12] and other microcrystalline diseases [18, 19]. Episodes of monoarticular, concomitant septic and crystalline arthritis were mostly reported in small case series [8, 12, 20, 21] or case reports. While many centres are still using Gram staining for a rapid, preliminary diagnosis of episodes of arthritis, the cost benefit of this approach has not been evaluated in detail.

In this single-centre study, we compared the sensitivities and specificities of Gram and acridine orange staining procedures [15] for detecting non-gonococcal arthritis in a prospective cohort of 500 episodes of arthritis. Besides evaluating the usefulness of these staining procedures for daily clinical practice, we tried to identify clinical variables that might help to predict positive stain results. In contrast, detailed therapeutic and pathophysiological aspects [3, 19] were not addressed in this study.

Methods

Setting and study design

The Geneva University Hospitals is a 2,000-bed tertiary hospital. Adult patients with a suspicion of septic arthritis are usually hospitalised in the Orthopaedic Surgery Service, which includes 132 acute care beds and a dedicated infectious diseases physician. The Orthopaedic Service also includes patients for hand surgery. All adult patients hospitalised for suspicion of septic arthritis, or prosthetic joint infection that underwent arthrocentesis, were included from January 1996 to 31 October 2012 until the 500th episode of arthritis was reached. The design of this study was approved by the local Ethics Committee.

Inclusion criteria and definitions

To avoid data clustering, only the first episode of arthritis was assessed. Patients' additional episodes were included only if they were clearly unrelated to the first one, e.g. by involving different joints in distinct time periods in a drug abuser. Immunosuppression was defined by the presence of active cancer, Child's class C cirrhosis, dialysis, untreated human immunodeficiency virus (HIV) disease, diabetes mellitus or receipt of chronic steroid medication. A concomitant bacteraemia was defined as a culture-positive blood pathogen similar to that found in synovial fluid.

Procedures

Synovial fluids were sent to the laboratory for evaluating white blood cell counts and differentials, crystal analysis, Gram and acridine orange staining, and microbiological cultures. Concomitant bacteraemia due to the same organism was evaluated by blood cultures. If there was a moderate or high suspicion of septic arthritis even in the presence of negative Gram and acridine orange stain results, therapeutic joint lavage by arthrotomy or arthroscopy (sometimes with synovial debridement) was initiated before receiving microbiological culture results. Furthermore, neutrophil differentials and crystal analysis by polarised microscopy were also evaluated in synovial fluid aspirates. Gram and acridine orange staining of synovial fluids were performed on centrifuged specimens. Identification of microbial pathogens was achieved according to Clinical and Laboratory Standards Institute's definitions [22]. By November 2012, overall cost estimates for Gram staining and acridine orange were 23 and US$29, respectively. The costs of the microbiological culture ranged from 65 (negative culture) to US$170 (positive culture with antimicrobial susceptibility testing).

Statistical analyses

Group comparisons were performed using either Pearson's χ^2 test or Wilcoxon rank sum test, as appropriate. Correlations were computed with Spearman's correlation. Unmatched logistic regression analyses determined associations with two outcome events: "positive Gram staining" (positive in terms of visible pathogens, not "Gram-positive" sensu stricto) and culture-positive arthritis. Independent variables with a p value ≤ 0.2 in univariate analysis were introduced stepwise in the multivariate analysis. We included approximately five to ten predictor variables per outcome event [23]. All variables were checked for confounding, colinearity and interaction, the latter by Mantel-Haenszel estimates and interaction terms. C-reactive protein (CRP) levels, the percentage of neutrophils and the overall number of synovial fluid leukocytes were analysed as continuous and categorised variables. The cut-off values in creating the categorisation strata were defined by the following criteria: The middle stratum was positioned around the median value, while the choice was made relying on the 33 and 66 percentiles of the distribution of values of that variable. The edges were rounded up to clinically realistic cut-off values. Finally, receiver-operating characteristic (ROC) curves determined the relationship of important variables with the outcome values. Two-tailed p values ≤ 0.05 were considered as significant. Stata software (9.0, Stata™, College Station, TX, USA) was used.

Results

Study population

In all, 500 episodes of arthritis were identified in 491 patients, including 208 women and 283 men (Table 1). A total of 196 patients had an underlying immunosuppression: diabetes mellitus (n=78), active cancer (n=36), Child's class C cirrhosis (n=11), HIV disease (n=10), dialysis (n=6) and solid organ transplantation (n=4). For 30 patients who received immunosuppressive steroid medication, the median dose was equivalent to 10 mg of prednisone daily (range 2.5–40 mg). The remaining patients had various immunosuppressed states or multiple immunosuppressions. Forty patients had documented gout and twelve rheumatoid polyarthritis [11]. Among the 38 different, documented origins of arthritis, surgical site infections (200) [24] and cutaneous origins (27) were the most frequent. Eleven patients were intravenous drug abusers. In 148 episodes, the origin remained unknown despite intensive workup [25]. In 105 cases, patients were already under 35 different antibiotic regimens during joint sampling.

Episodes of septic arthritis

The knee was the most frequently affected joint (192/500 episodes, 38 %), followed by the hip (146), shoulder (54) and ankle (23); 227 episodes were associated with prosthetic joints. Seven patients had two independent episodes of arthritis occurring in different time periods, and two patients had three episodes. The median CRP level at joint sampling was 132 mg/l (interquartile range 59–249 mg/l). This CRP value was higher among the 128 documented bacteraemic compared to non-bacteraemic infectious episodes (p <0.01). The median synovial leukocyte count was 40,629 cells/mm^3 (interquartile range 16,000–88,000 cells/mm^3). The median percentage of neutrophils was 90 % (interquartile range 85–94 %).

Pathogens and crystals

Synovial fluids were culture positive in 400 of the 500 episodes of arthritis (80 %). Among the 61 retrieved different pathogens [26], *Staphylococcus aureus* was the most frequent (n=170), followed by various streptococci (73) and Gram-negative rods (61). Thirty-five infections were polymicrobial. Synovial fluid examination revealed the presence of monosodium urate crystals in 23 episodes (5 %), calcium pyrophosphate crystals in 43 episodes (9 %) and apatite crystals in 5 episodes (1 %). Sodium urate and pyrophosphate crystals were concomitantly found in three episodes. Among 69 episodes with crystalline disease, synovial fluid cultures also grew pathogens in 42 cases. This was the case for 12 episodes of gout, 24 episodes of pseudogout (chondrocalcinosis), 1 episode of mixed urate-pyrophosphate arthritis and 5 episodes of apatite-related

Table 1 Characteristics of patient populations with the detection of pathogens in Gram stain versus no detection

All episodes of arthritis	Total n=500	Gram staining with pathogens n=146	Comparison p value[a]	Gram staining without pathogens n=354
Female gender	208	68 (47 %)		140 (40 %)
Median CRP level		206 mg/l	0.001	104 mg/l
Median leukocyte count in synovial fluid		82,500 cells/mm^3	0.001	33,954 cells/mm^3
Median % of neutrophils in synovial fluid		91 %		90 %
Immunosuppression[b]	196	67 (46 %)	0.049	129 (36 %)
Diabetes mellitus	78	26 (18 %)		52 (15 %)
Steroid medication	30	15 (10 %)	0.010	15 (4 %)
Patient known for gout	40	11 (8 %)		29 (8 %)
Patient known for rheumatoid arthritis	14	6 (4 %)		8 (2 %)
Underlying arthroplasty	227	66 (45 %)		161 (45 %)
Hip arthritis	146	37 (25 %)		109 (31 %)
Knee arthritis	192	61 (42 %)		131 (37 %)
Finger/toe arthritis	33	8 (5 %)		25 (7 %)
Bacteraemia	128	67 (46 %)	0.001	61 (17 %)
Under antibiotic therapy	105	30 (21 %)		75 (21 %)
Presence of crystals in synovial fluid[c]	69	23 (16 %)		46 (13 %)

[a] Only significant p values ≤0.05 (two-tailed) are displayed; Pearson's χ^2 test or Wilcoxon rank sum test, as appropriate
[b] Immunosuppression=diabetes mellitus, steroids, transplantation, untreated HIV infection, dialysis, Child's class C cirrhosis, active cancer
[c] Presence of sodium urate, pyrophosphate or apatite crystals in the microscopic examination of sampled synovial fluid

disease. Of note, in 17 of 69 cases, crystal-associated arthritis involved arthroplasty joints.

Performance and costs of Gram and acridine orange stains

Gram staining performed of the 500 episodes of arthritis revealed pathogens in only 146 episodes (29 %). The ability of Gram stain for detecting the 400 culture-positive episodes was 37 % (146/400). For 138 of those 146 episodes (95 %), Gram staining and microbiological cultures yielded the same pathogen. For only six episodes (5 %) was species identification by the Gram stain different from that by microbiological culture. The performance of acridine orange and Gram staining procedures was quite similar. It was performed in 398 episodes and yielded pathogens in 100 episodes (100/398, 25 %) or in 100 of 400 culture-positive episodes (25 %) (Spearman correlation 0.85, $p<0.001$). We further evaluated the sensitivity, specificity, positive predictive value and negative predictive value of Gram and acridine orange staining for predicting culture-positive results in septic arthritis, for either the overall population or the different subgroups of patients (Table 2).

In brief, the sensitivities and negative predictive values ranged between 0.16 and 0.52, whereas the specificity and positive predictive values were excellent. These staining procedures were relatively expensive. For culture-negative cases, Gram and acridine staining procedures represented 45 % of all laboratory costs, but only 25 % for culture-positive cases. Overall, among the 500 episodes, total estimated expenditures for the staining work amounted to US$22,513 (status November 2012).

Logistic regression analyses

Due to differences in the crude group comparisons (Tables 1 and 2) as well as statistically significant associations in univariate analyses (Tables 3 and 4), multivariate analyses were performed to adjust for the substantial case mix. These analyses were performed by using as outcome bacterial detection by Gram stain (Table 3) or alternatively by microbiological culture (Table 4). In multivariate analyses, several host parameters were significantly linked with positive Gram stain results, in particular steroid medication, CRP levels>150 mg/l and synovial leukocyte counts>180,000 cells/mm^3. In

Table 2 Stratification by two by two tables for the predilection of culture-positive septic arthritis

All episodes of arthritis	Culture positive	Culture negative	Total
Gram stain showing pathogens	145	1	146
Gram stain without pathogens	255	99	354
Total	400	100	500
Sensitivity 0.37, specificity 0.99, positive predictive value 0.99, negative predictive value 0.28			
Arthroplasty infections	Culture positive	Culture negative	Total
Gram stain showing pathogens	66	0	66
Gram stain without pathogens	135	26	161
Total	201	26	227
Sensitivity 0.33, specificity 1.00, positive predictive value 1.00, negative predictive value 0.16			
Concomitant antibiotic therapy	Culture positive	Culture negative	Total
Gram stain showing pathogens	28	0	28
Gram stain without pathogens	50	27	77
Total	78	27	105
Sensitivity 0.36, specificity 1.00, positive predictive value 1.00, negative predictive value 0.35			
Immunosuppression[a]	Culture positive	Culture negative	Total
Gram stain showing pathogens	67	0	67
Gram stain without pathogens	97	32	129
Total	164	32	196
Sensitivity 0.40, specificity 1.00, positive predictive value 1.00, negative predictive value 0.25			
Concomitant synovial crystals[b]	Culture positive	Culture negative	Total
Gram stain showing pathogens	22	1	23
Gram stain without pathogens	20	26	46
Total	42	27	69
Sensitivity 0.52, specificity 0.96, positive predictive value 0.96, negative predictive value 0.56			

[a] Immunosuppression=diabetes mellitus, steroids, transplantation, untreated HIV infection, dialysis, Child's class C cirrhosis, active cancer
[b] Presence of sodium urate, pyrophosphate or apatite crystals in the microscopic examination of sampled synovial fluid

Table 3 Variables associated with a positive Gram staining result (unmatched logistic regression)

	Univariate analysis		Multivariate analysis	
All episodes of arthritis	Odds ratio (95 % confidence interval)	*p* value[a]	Odds ratio (95 % confidence interval)	*p* value[a]
Female gender	1.3 (0.9–1.7)		–	
CRP level (continuous variable)	1.001 (1.003–1.006)	<0.001	1.009 (1.004–1.013)	<0.001
CRP ≤80 mg/l	1		1	
CRP >80 to ≤150 compared to ≤80 mg/l	1.4 (0.8–2.7)		1.5 (0.8–2.9)	
CRP >150 compared to ≤80 mg/l	2.5 (1.5–4.0)	<0.001	2.5 (1.5–3.9)	<0.001
Patient known for gout	0.9 (0.4–1.9)		–	
Patient known for rheumatoid polyarthritis	1.9 (0.6–5.4)		–	
Immunosuppression[b]	1.5 (1.0–2.2)	0.050	–	
Diabetes mellitus	1.3 (0.8–2.1)		–	
Steroid medication	2.6 (1.2–5.4)	0.012	2.2 (0.9–4.8)	
Dose of steroids (continuous variable)	1.08 (1.02–1.14)	0.008	1.1 (1.002–1.2)	0.044
Synovial leukocyte count (continuous variable)	1.0 (0.9–1.1)		1.0 (1.0–1.0)	
Synovial leukocyte count ≤90,000 cells/mm^3	1		1	
Leukocyte count >90,000–180,000 cells/mm^3	4.8 (1.5–15.4)	0.008	3.4 (0.9–12.0)	
Leukocyte count >180,000 cells/mm^3	2.6 (1.4–4.7)	0.002	4.5 (1.8–10.8)	0.001
Knee arthritis	1.2 (0.8–1.8)		–	
Hip arthritis	0.8 (0.5–1.2)		–	
Large joint arthritis[c]	0.7 (0.5–1.1)		–	
Finger/toe arthritis	0.8 (0.3–1.7)		–	
Bacteraemia	3.6 (2.3–4.9)	<0.001	–	
Gram-positive pathogens	3.1 (1.5–6.1)	0.001	–	
Under antibiotic therapy	0.9 (0.6–1.5)		–	
Presence of crystals in synovial fluid	2.0 (1.0–4.0)		1.2 (0.4–4.0)	
Quantity of crystals ++ compared to +	1.6 (0.4–5.4)		–	
Quantity of crystals +++ compared to +	1.8 (0.7–5.0)		–	
Arthroplasty infection	1.0 (0.7–1.5)		–	
Percentage of neutrophils in synovial fluid	1.0 (0.9–1.1)		1.0 (0.9–1.1)	
Neutrophils ≤85 %	1		1	
Neutrophils >85 % to ≤95 %	2.2 (0.7–6.6)		1.5 (0.5–5.0)	
Neutrophils >95 %	1.9 (0.7–5.2)		0.7 (0.2–2.2)	

[a] Only significant *p* values ≤0.05 (two-tailed) are displayed
[b] Immunosuppression=diabetes mellitus, steroids, transplantation, untreated HIV infection, dialysis, Child's class C cirrhosis, active cancer
[c] Large joint arthritis=hip, knee, elbow, ankle

contrast, other parameters such as the presence of underlying prosthetic joints, the size of articulation, gender, ongoing antibiotic therapy or the presence of crystals were not significantly linked with bacterial detection by Gram staining (Table 3). Among variables associated with a positive microbiological joint culture, the same variables were associated with a positive culture, plus a positive Gram result. In multivariate analyses, identification of pathogens by Gram staining was strongly predictive of positive culture results with an odds ratio of 32, whereas the presence of crystals only slightly excluded a microbiologically positive culture (Table 4). Both final models were valid. The goodness-of-fit tests were insignificant; the ROC value was 0.82 and 0.83, respectively.

ROC curves

The association of the outcome bacterial detection by Gram stain with three clinical variables (CRP levels, synovial leukocyte differentiation and percentage of synovial neutrophils) was evaluated by ROC curves (Figs. 1, 2 and 3). The association was ubiquitously poor, without any visible and obvious thresholds in favour of prediction of the outcome. For example, regarding a threshold for a CRP level of 335 mg/l, the sensitivity was 30 %, but the specificity was 90 %. By diminishing the threshold to 30 mg/l, the sensitivity increased to 95.7 %, but the specificity decreased to 17.7 %. Likewise, for the synovial leukocyte

Table 4 Variables associated with culture-positive septic arthritis (unmatched logistic regression)

	Univariate analysis		Multivariate analysis	
All episodes of arthritis	Odds ratio (95 % confidence interval)	*p* value[a]	Odds ratio (95 % confidence interval)	*p* value[a]
Female gender	0.8 (0.5–4.7)		–	
CRP level (continuous variable)	1.004 (1.002–1.006)	<0.001	1.0 (1.0–1.0)	
CRP ≤80 mg/l	1		1	
CRP >80 to ≤150 compared to ≤80 mg/l	0.9 (0.5–1.5)		–	
CRP >150 compared to ≤80 mg/l	2.7 (1.6–4.5)	<0.001	–	
Immunosuppression[b]	1.5 (0.9–2.4)		–	
Diabetes mellitus	1.6 (0.8–3.2)		–	
Steroid medication	1.7 (0.6–4.9)		0.6 (0.1–3.3)	
Synovial leukocyte count (continuous variable)	1.0 (0.9–1.1)			
Synovial leukocyte count ≤90,000 cells/mm^3	1		1	
Leukocyte count >90,000–180,000 cells/mm^3	2.2 (0.6–7.2)		0.8 (0.1–4.5)	
Leukocyte count >180,000 cells/mm^3	4.7 (2.9–7.8)	<0.001	3.9 (0.8–20.3)	
Knee arthritis	0.7 (0.5–1.1)		0.8 (0.2–2.6)	
Finger/toe arthritis	0.5 (0.2–1.1)		–	
Bacteraemia	10.1 (3.1–32.9)	<0.001	–	
Under antibiotic therapy	0.7 (0.4–1.2)		0.9 (0.2–2.6)	
Presence of pathogens in Gram staining	56.3 (7.8–407.8)	<0.001	32.3 (3.5–301.7)	0.002
Presence of crystals in synovial fluid	0.5 (0.2–0.9)	0.026	0.3 (0.1–0.8)	0.021
Percentage of neutrophils in synovial fluid	1.0 (0.9–1.1)		1.0 (0.9–1.1)	
Neutrophils ≤85 %	1		–	
Neutrophils >85 % to ≤95 %	2.1 (0.8–5.2)		–	
Neutrophils >95 %	5.3 (2.4–11.6)	<0.001	–	

[a] Only significant *p* values ≤0.05 (two-tailed) are displayed

[a] Immunosuppression=diabetes mellitus, steroids, transplantation, untreated HIV infection, dialysis, Child's class C cirrhosis, active cancer

counts, a threshold of 22,000 cells/mm^3 yielded a sensitivity of 100 % and a specificity of 39 %. By raising the threshold to 150,000 cells/mm^3, sensitivity decreased to 16.7 %, and specificity increased to 90.2 %.

Discussion

In our large cohort study, the sensitivity of Gram or acridine orange stains for predicting culture-positive, septic arthritis

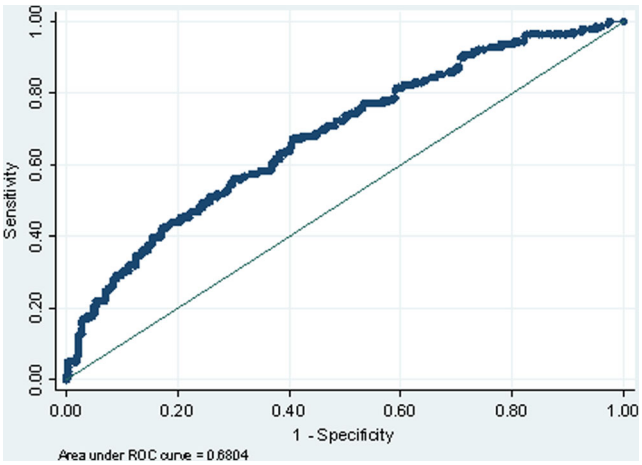

Fig. 1 ROC curve. CRP levels against the prediction of culture-positive septic arthritis

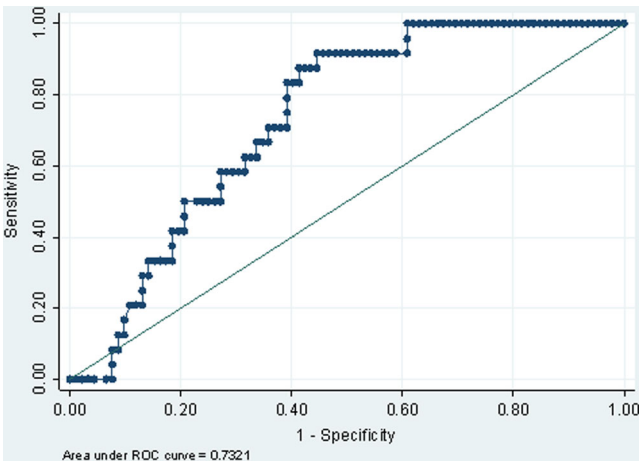

Fig. 2 ROC curve. Synovial leukocyte count levels against the prediction of culture-positive septic arthritis

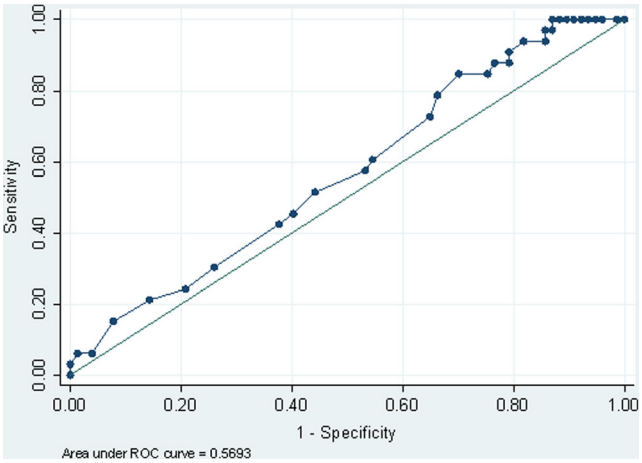

Fig. 3 ROC curve. Percentage levels of synovial neutrophils against the prediction of culture-positive septic arthritis

was of questionable value [17], independently of the presence of underlying material, immunosuppression and ongoing antibiotic or steroid therapy. The sensitivity in the presence of synovial fluid crystals is moderate, equally precluding the possibility of excluding infection or a positive Gram stain result whenever crystals are present [8, 12, 20]. Acridine orange revealed the same results as Gram stain and is slightly more expensive than the latter. In terms of cost-effectiveness, neither staining can be considered as mandatory for the diagnosis of septic arthritis. Hence, in the current situation, the therapeutic decision for a suspected episode of septic arthritis is still guided by the history and clinical presentation of patients combined with the experience of physicians and surgeons.

In multivariate analyses, the severity of the joint inflammation and underlying immunosuppression were both associated with the probability of detecting pathogens by Gram staining. A CRP level above 150 mg/l, bacteraemia and a synovial leukocyte count above 180,000 cells/mm^3 were also significantly linked with the outcome positive Gram staining, but not the percentage of neutrophil counts in synovial fluid, the presence of underlying prosthetic joints, size of articulation, gender, ongoing antibiotic therapy or the presence of crystals. Among the large, heterogeneous group of immunocompromised patients, those receiving concomitant steroid medication were the most significantly associated with early bacterial detection by Gram stain.

In the literature, the overall sensitivity ranges between 30 and 60 % [1, 3, 7], whereas for prosthetic joint infections, Morgan et al. identified a 27 % sensitivity of intraoperative Gram staining [17], while the value of inflammatory markers for predicting culture-positive arthritis was less convincing. Several studies mentioned a positive association between occurrence of septic arthritis and elevated serum CRP levels [17]. Ernst et al. showed that a high CRP predicted a septic joint [14]. Hariharan and Kabrhel reported a linear relationship between CRP level and occurrence of septic arthritis, yielding a sensitivity of ca. 45 % at a cut-off of 150 mg/l in a group of 167 patients [16]. Papanicolas et al. identified a CRP >100 mg/l as predictive of septic arthritis with a sensitivity of 86 %, but a low specificity of 48 % [20]. Other reports challenged the predictive value of serum inflammatory markers for the diagnosis of septic arthritis [7]. While our study detected a significant association of a CRP value above 150 mg/l as "predictive" in multivariate analysis, the corresponding ROC curve was moderate at best.

Conflicting data were provided on the significance of synovial leukocyte counts. Most experts consider that a threshold of 50,000 cells/mm^3 is particularly predictive of a septic origin. McGillicuddy et al. attributed 61 % sensitivity to a cut-off level of 50,000 cells/mm^3 [3, 6]. Other groups proposed higher thresholds up to 100,000 cells/mm^3 [2]. In reality, different studies reveal different sensitivity results at different cut-off levels, while for other groups even 100,000 leukocytes cannot reliably discriminate septic from non-septic arthritis [4]. Again, our ROC curve failed to reveal any particularly useful threshold for the association of synovial leukocyte counts with a positive aspiration staining.

Regarding the significance of neutrophil differentials in synovial fluid, a literature review involving 6,242 patients indicated that a neutrophil proportion above 90 % had a threefold likelihood for septic arthritis compared to less than 90 % [1]. This cut-off was set at 75 % by other authors [3]. In our study, neutrophil differentials showed a statistical relationship with culture-positive bacterial arthritis only at a cut-off of 95 %, whereas the ROC curve yielded a very poor association.

Despite including the largest number of episodes of septic arthritis in the literature, our study has limitations: (1) It was performed in a single institution with a very heterogeneous adult patient population, aspects that limit extrapolation of its findings. (2) The design of our study reflected a real-life situation and hence did not address potential innovative microbiological techniques in the rapid diagnostics of septic arthritis, such as the use of polymerase chain reactions [7, 26], synovial fluid glucose [1, 3, 7], proteins [1, 3], lactic acid [7, 9], serum erythrocyte sedimentation rate (ESR) [1, 7, 14, 16], lactate dehydrogenase [1], various interleukins [7] or procalcitonin levels [10, 13, 27], which may potentially help to discriminate septic from non-septic arthritis.

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