Synovial Fluid Leukocyte Count and Differential for the Diagnosis of Prosthetic Knee Infection

Andrel Trampuz, MD, Arlen D. Hanssen, MD, Douglas R. Osmon, MD, MPH, Jayawant Mandrekar, PhD, James M. Steckelberg, MD, Robin Patel, MD

PURPOSE: Criteria for the interpretation of synovial fluid are well established for native joint disorders but lacking for the evaluation of prosthetic joint failure. Our aim was to define cutoff values for synovial fluid leukocyte count and neutrophil percentage for differentiating aseptic failure and prosthetic joint infection.

METHODS: We performed a prospective study of 133 patients in whom synovial fluid specimens were collected before total knee arthroplasty revision between January 1998 and December 2003. Patients with underlying inflammatory joint disease were excluded.

RESULTS: Aseptic failure was diagnosed in 99 patients and prosthetic joint infection was diagnosed in 34 patients. The synovial fluid leukocyte count was significantly higher in patients with prosthetic joint infection (median, 18.9 × 10^3/µL; range, 0.3 to 178 × 10^3/µL) than in those with aseptic failure (median, 0.3 × 10^3/µL; range, 0.1 to 16 × 10^3/µL; P < 0.0001); the neutrophil percentage was also significantly higher in patients with prosthetic joint infection (median [range], 92% [55% to 100%] vs. 7% [0% to 79%], P < 0.0001). A leukocyte count of >1.7 × 10^3/µL had a sensitivity of 94% and a specificity of 88% for diagnosing prosthetic joint infection; a differential of >65% neutrophils had a sensitivity of 97% and a specificity of 98%. Staphylococcus aureus was the only pathogen associated with leukocyte counts >100 × 10^3/µL.


Examination of synovial fluid is a fundamental diagnostic tool in the evaluation of native joint disorders (1–4). Routine analysis usually includes a leukocyte count and differential, Gram stain and culture, and examination for crystals (5). In 1953, Ropes and Bauer classified synovial fluid based on gross appearance and leukocyte count into noninflammatory (group 1) and inflammatory (group 2) categories, correlating them with various disease entities (6). Later, visible purulent exudates were classified as a separate, "septic" category (group 3) (7). Although the criteria for the interpretation of synovial fluid leukocyte count and differential are well established for native joint disorders, there are no established criteria for the evaluation of prosthetic joint failure (8–13).

The number of joint replacement surgeries performed has been increasing steadily since arthroplasty was first performed (14). Aseptic loosening is the most common cause of prosthetic joint failure, followed by infection. Differentiation of these two entities is important because their management differs (15,16). Synovial fluid cultures are frequently used to diagnose prosthetic joint infection. However, culture methods usually require several days to identify the presence (or absence) of microorganisms, and certain microorganisms may be difficult to detect. Furthermore, cultures may be subject to contamination. A simple, rapid, and accurate test for differentiating prosthetic joint infection from aseptic failure would be helpful, especially if the procedure could be performed preoperatively and in the outpatient setting. We therefore sought to determine the optimal cutoff values of synovial fluid leukocyte count and neutrophil percentage for the diagnosis of prosthetic joint infection in patients without underlying inflammatory joint disease.

METHODS

Patient Selection and Definitions
A prospective study was performed on patients with a total knee arthroplasty who had undergone synovial fluid aspiration for preoperative evaluation of arthroplasty failure at Mayo Clinic, Rochester, between January 1998 and December 2003. Patients with underlying inflammatory joint diseases (e.g., rheumatoid arthritis, psoriatic arthritis), crystal-induced arthropathy, or connective tissue diseases were excluded. Each patient was included only once. Medical records of study subjects were abstracted for demographic information and data related to synovial fluid and arthroplasty surgery. Antimicrobial treatment was defined as receipt of any antimicrobial...
agent for >1 month and administered through at least 2 weeks before arthrocentesis. Patients were classified as having either aseptic failure or prosthetic joint infection based on preoperative and intraoperative findings using a previously described classification system (17). Prosthetic joint infection was diagnosed if at least one of the following criteria was present: growth of the same microorganism in at least two cultures of synovial fluid or periprosthetic tissue; visible synovial fluid purulence at the time of arthrocentesis or during surgery; acute inflammation on histopathologic examination of permanent periprosthetic tissue sections (as determined by the clinical pathologist); or presence of a sinus tract communicating with the prosthesis. A causative pathogen was defined as a microorganism cultured from synovial fluid or periprosthetic tissue in a patient with prosthetic joint infection. Aseptic failure was defined as prosthetic failure not meeting the criteria for prosthetic joint infection. The study was approved by the clinic’s institutional review board, and all patients gave informed consent.

**Arthrocentesis and Synovial Fluid Analysis**

After skin preparation and local anesthesia with 1% lidocaine, synovial fluid was aseptically collected using a sterile 18-gauge spinal needle and a 10-mL syringe. If no synovial fluid was obtained initially, the needle was repositioned without withdrawing it through the skin; no fluid was injected into the joint. The clinician noted the volume of synovial fluid and the presence or absence of purulence. The aspirate was transferred into two vials: one for cell counting containing ethylenediaminetetraacetic acid (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, New Jersey) and the other for culture (without additives). The vials were transported to the laboratory within 6 hours. The appearance of the synovial fluid was described by the laboratory technician as serous, cloudy, or bloody. Clotted specimens were treated with hyaluronidase (Sigma, St. Louis, Missouri) for 10 minutes at room temperature. Leukocyte count and differential were determined by microscopic examination. For culture, 0.1 mL of synovial fluid was inoculated to each of the following: trypticase soy blood agar, chocolate blood agar, anaerobic blood agar, and thioglycollate broth (BD Diagnostic Systems, Sparks, Maryland). Aerobic cultures were incubated for 5 days and anaerobic cultures were incubated for 7 days. Residual volumes of synovial fluid >0.5 mL were also inoculated into a BACTEC Peds Plus/F bottle and incubated in a BACTEC 9240 instrument (BD Diagnostic Systems) for 5 days.

**Statistical Analysis**

Variables were compared using the chi-squared test, Fisher exact test, or Wilcoxon rank sum test, as appropriate. The optimal cutoff values of synovial fluid leukocyte count and neutrophil percentage for diagnosing prosthetic joint infection were determined using a previously described method (18). For the identified cutoff values, the sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were calculated using 2 × 2 contingency tables.

The accuracy of synovial fluid leukocyte count and differential as diagnostic markers was evaluated by constructing receiver operating characteristic (ROC) curves, which depict the relation between true-positive (sensitivity) and false-positive (1 - specificity) test results. Areas under the ROC curves were calculated using the method of Hanley and McNeil (19) and compared using the method of DeLong et al (20). Spearman rank correlation coefficient (r_s) was used to study the strength of the association between synovial fluid leukocyte and neutrophil percentage. The association between prosthetic joint infection as a binary response variable and synovial fluid leukocyte count and neutrophil percentage as explanatory variables was assessed univariately using logistic regression (21).

A P value <0.05 (two-sided) was considered statistically significant. All calculations were performed using SAS statistical software package, version 8.2 (SAS Institute Inc., Cary, North Carolina). For graphic analysis, Origin software, version 7.5 (OriginLab Corp., Northampton, Massachusetts), was used.

**RESULTS**

A total of 133 synovial fluid specimens from 78 men and 55 women (median age, 71 years; range, 26 to 99 years) with total knee arthroplasties were studied. In all subjects, the prosthesis had been implanted more than 6 months before arthrocentesis. Ninety-nine patients had aseptic failure and 34 patients had prosthetic joint infection (Table 1). The causative microorganism was found in the synovial fluid or tissue cultures of 31 patients (91%) with prosthetic joint infection and included coagulase-negative staphylococci (n = 16), *Staphylococcus aureus* (n = 8), viridans group streptococci (n = 2), *Propionibacterium acnes* (n = 2), *Corynebacterium jeikeium* (n = 1), *Pseudomonas aeruginosa* (n = 1), and *Enterococcus* species (n = 1). Synovial fluid cultures yielded the causative microorganism in 77% [26/34] of cases and periprosthetic tissue cultures in 88% [22/25] of cases with prosthetic joint infection. A sinus tract communicating with the prosthesis was present in 3 subjects with prosthetic joint infection: *S. aureus* was the causative microorganism in 2 subjects and *P. aeruginosa* in 1. In patients with aseptic failure, coagulase-negative staphylococci (n = 4), *C. jeikeium* (n = 1), and *P. acnes* (n = 1) grew in a single culture specimen.

**Synovial Fluid Appearance and Cell Count**

Cloudy synovial fluid was observed in 38% of patients with prosthetic joint infection and in 16% of patients with aseptic failure (Table 1). The synovial fluid leuko-
Cytes were signifi-
cantly higher in patients with prosthetic joint infection (median, 18.9 \times 10^9/\mu L; range, 0.3 to 178 \times 10^9/\mu L) than in those with aseptic failure (median, 0.3 \times 10^9/\mu L; range, 0.1 to 16 \times 10^9/\mu L; P < 0.0001; Figure 1). Similarly, the neutrophil percentage was significantly higher in patients with prosthetic joint infection (median, 92%; range, 55% to 100%) than in those with aseptic failure (median, 7%; range, 0% to 79%; P < 0.0001). Synovial fluid leukocyte count and neutrophil percentage were significantly correlated with one another (r_s = 0.53, P < 0.0001).

Univariate logistic regression analysis revealed an association between prosthetic joint infection and leukocyte count (odds ratio [OR] per increase of 1 \times 10^9/\mu L = 1.50; 95% confidence interval [CI]: 1.24 to 1.82), as well as between prosthetic joint infection and neutrophil percentage (OR per increase of 1% = 1.20; 95% CI: 1.09 to 1.31). ROC curves were used for comparing the sensitivity and specificity of synovial fluid leukocyte count and neutrophil percentage differentiating aseptic failure from prosthetic joint infection (Figure 2). Incremental increases in true-positive rates (sensitivity) were associated with relatively smaller increases in false-positive rates (1 - specificity) for neutrophil percentage as compared with leukocyte count, indicating a better diagnostic accuracy for neutrophil percentage. The area under the ROC curve was 0.96 (95% CI: 0.94 to 0.98) for leukocyte count and 0.997 (95% CI: 0.991 to 0.999) for neutrophil percentage (P = 0.02).

The cutoff values for optimal sensitivity and specificity to differentiate aseptic failure from prosthetic joint infection were 1.7 \times 10^9/\mu L for synovial fluid leukocyte count and 65% for neutrophil percentage (Table 2). A leukocyte count of > 1.7 \times 10^9/\mu L had a sensitivity of 94% and a specificity of 88% for diagnosing prosthetic joint infection, whereas a differential of > 65% neutrophils had a sensitivity of 97% and a specificity of 98%. Applying cutoff values for diagnosing septic arthritis in native joints

Table 1. Characteristics of 133 Patients with Total Knee Arthroplasty Failure*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aseptic Failure (n = 99)</th>
<th>Prosthetic Joint Infection (n = 34)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%) or Median (Range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>71 (40–99)</td>
<td>72 (26–90)</td>
<td>0.35</td>
</tr>
<tr>
<td>Women</td>
<td>40 (40)</td>
<td>15 (44)</td>
<td>0.70</td>
</tr>
<tr>
<td>Failed prosthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary arthroplasty</td>
<td>64 (64)</td>
<td>24 (71)</td>
<td>0.53</td>
</tr>
<tr>
<td>Fixation with bone cement</td>
<td>87 (88)</td>
<td>31 (91)</td>
<td>0.76</td>
</tr>
<tr>
<td>Duration from arthroplasty to joint aspiration (months)</td>
<td>28.4 (6.1–185.3)</td>
<td>21.5 (6.3–105.5)</td>
<td>0.19</td>
</tr>
<tr>
<td>Duration of clinical symptoms (months)†</td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>&lt;1</td>
<td>11 (11)</td>
<td>5 (15)</td>
<td></td>
</tr>
<tr>
<td>1–12</td>
<td>53 (54)</td>
<td>22 (65)</td>
<td></td>
</tr>
<tr>
<td>&gt;12</td>
<td>35 (35)</td>
<td>7 (21)</td>
<td></td>
</tr>
<tr>
<td>Synovial fluid characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visible purulence noted by the surgeon‡</td>
<td>0</td>
<td>16 (47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gross appearance noted by the laboratory technician</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>60 (61)</td>
<td>8 (24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cloudy</td>
<td>16 (16)</td>
<td>13 (38)</td>
<td>0.01</td>
</tr>
<tr>
<td>Bloody</td>
<td>23 (23)</td>
<td>13 (38)</td>
<td>0.09</td>
</tr>
<tr>
<td>Positive synovial fluid culture</td>
<td>1 (1)</td>
<td>26 (77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leukocytes (\times 10^9/\mu L)§</td>
<td>0.3 (0.1–16)</td>
<td>18.9 (0.3–178)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutrophils (%)§</td>
<td>7 (0–79)</td>
<td>92 (55–100)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Where the denominator is shown, data were not available for all study subjects. Percentages have been rounded.
† Joint pain, effusion, erythema, warmth of the skin overlying the device, or sinus tract communicating with the implant.
‡ Considered a diagnostic criterion for prosthetic joint infection.
§ Data are represented in Figure 1.

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(>50 × 10^3/µL for leukocytes and >90% for neutrophils) (8), the sensitivity for diagnosing prosthetic joint infection was only 21% for leukocytes and 59% for neutrophils.

**Type of Microorganism and Antimicrobial Treatment**

The distribution of synovial fluid cell counts varied by type of microorganism causing prosthetic joint infection (Figure 3). Patients receiving antimicrobial agents (n = 14) had lower leukocyte counts (median, 12.1 × 10^3/µL; range, 1.9 to 68.8 × 10^3/µL) than those who were not receiving antimicrobial agents (median, 33.3 × 10^3/µL; range, 0.3 to 178.1 × 10^3/µL; P < 0.001). For patients who had not received antimicrobial treatment, *S. aureus* was associated with leukocyte counts >100 × 10^3/µL, while coagulase-negative staphylococci were associated with counts <50 × 10^3/µL. *P. acnes* and *C. jeikeium* were associated with leukocyte counts <10 × 10^3/µL. No correlation was observed between neutrophil percentage and microorganism type or antimicrobial treatment.

**DISCUSSION**

We found that a synovial fluid leukocyte differential of >65% neutrophils (or a leukocyte count of >1.7 × 10^3/µL) was the optimal cutoff value for identifying patients with prosthetic joint infection. These cutoff values are considerably lower than those used to diagnose infection in native joints, probably reflecting the lower virulence and biofilm phenotype of microorganisms causing prosthetic joint infection (4,5,8–11).

High cutoffs for synovial fluid cell counts suggested in previous studies explain their low sensitivity for diagnosing prosthetic joint infection. For example, cutoffs for leukocytes >25 × 10^3/µL and >75% neutrophils have been proposed by several authors for predicting prosthetic joint infection in patients with hip or knee arthroplasties (22–25). Applied to our study sample, the sensitivity of a synovial fluid leukocyte cutoff of >25 × 10^3/µL for the diagnosis of prosthetic joint infection would be just 44%. Other authors have recommended an even
higher cutoff of $>80 \times 10^9/\mu L$, which, if applied to our study sample, would have a sensitivity of 11% (26). In agreement with these findings, Spangehl et al reported a sensitivity of 36% and specificity of 99% for diagnosing infection associated with hip arthroplasties when a cutoff of $>50 \times 10^9/\mu L$ was used (27). Kersey et al evaluated patients with aseptic knee prosthesis failure and found a mean synovial fluid leukocyte count of $0.78 \times 10^9/\mu L$ (range, 0.01 to $7.2 \times 10^9/\mu L$) and neutrophil percentage of 13% (range, 0% to 100%) (28). Mason et al recently found a significantly lower leukocyte count (0.65 vs. 25.9 $\times 10^9/\mu L$) and neutrophil percentage (27% vs. 73%) in patients with aseptic knee prosthesis failure than in those with infected knee prostheses (29). This retrospective study, however, did not evaluate the effects of underlying inflammatory joint diseases, antimicrobial therapy, and type of infecting organism, and the technique of synovial fluid analysis was not reported. These issues were addressed in our study.

Our study demonstrates that if optimal cutoff values are used, synovial fluid leukocyte count and neutrophil percentage have a comparable sensitivity and specificity for diagnosing prosthetic joint infection as intraoperative tissue culture and histopathology, which are frequently used as reference standard diagnostic markers. Our results also suggest that antimicrobial therapy may suppress the inflammatory response in the joint (Figure 3); however, no patient receiving antimicrobial therapy demonstrated synovial fluid leukocyte counts of $\leq 1.7 \times 10^9/\mu L$. Therefore, antimicrobial treatment did not decrease the sensitivity of leukocyte counts in diagnosing prosthetic joint infection. The synovial fluid leukocyte count was also associated with the type of microorganism, probably reflecting differences in microbial virulence. Gross appearance of the synovial fluid had little value in the assessment of the reason for prosthetic joint failure.

Besides good discriminatory ability, synovial fluid examination offers other advantages. It can be performed preoperatively and in the outpatient setting, providing valuable information with a turnaround time of a few hours (30). Early detection of prosthetic joint infection may improve outcomes by enabling appropriate surgical planning and early antimicrobial treatment. Compared with tissue histopathologic examination, synovial fluid cell count analysis is more

### Table 2. Comparison of Cutoff Values for Synovial Fluid Leukocyte Count and Neutrophil Percentage for Predicting Prosthetic Joint Infection *

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
<th>Positive Likelihood Ratio</th>
<th>Negative Likelihood Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoffs with optimal combination of sensitivity and specificity for diagnosing prosthetic joint infection in current report</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes $&gt;1.7 \times 10^9/\mu L$</td>
<td>94 (80-99)</td>
<td>88 (80-93)</td>
<td>73 (57-85)</td>
<td>98 (92-100)</td>
<td>8 (5-13)</td>
<td>0.1 (0.0-0.3)</td>
</tr>
<tr>
<td>Neutrophils $&gt;65%$</td>
<td>97 (85-100)</td>
<td>98 (93-100)</td>
<td>94 (81-99)</td>
<td>99 (95-100)</td>
<td>48 (12-190)</td>
<td>0.0 (0.0-0.2)</td>
</tr>
<tr>
<td>Cutoffs used for diagnosing inflammatory joint disorders in native joints*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Leukocytes $&gt;2 \times 10^9/\mu L$</td>
<td>91 (76-98)</td>
<td>89 (81-94)</td>
<td>74 (58-86)</td>
<td>97 (91-99)</td>
<td>8 (5-15)</td>
<td>0.1 (0.0-0.3)</td>
</tr>
<tr>
<td>Neutrophils $&gt;75%$</td>
<td>94 (80-99)</td>
<td>98 (93-100)</td>
<td>94 (80-99)</td>
<td>98 (93-100)</td>
<td>47 (12-184)</td>
<td>0.1 (0.0-0.2)</td>
</tr>
<tr>
<td>Cutoffs used for diagnosing septic arthritis in native joints*</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes $&gt;50 \times 10^9/\mu L$</td>
<td>21 (9-38)</td>
<td>100 (96-100)</td>
<td>100 (59-100)</td>
<td>79 (70-85)</td>
<td>$\infty$</td>
<td>0.8 (0.7-0.9)</td>
</tr>
<tr>
<td>Neutrophils $&gt;90%$</td>
<td>59 (41-75)</td>
<td>100 (96-100)</td>
<td>100 (83-100)</td>
<td>88 (80-93)</td>
<td>$\infty$</td>
<td>0.4 (0.3-0.6)</td>
</tr>
</tbody>
</table>

* The 95% confidence intervals were calculated as exact binomial confidence intervals. Percentages have been rounded. The prevalence of prosthetic joint infection in our study sample was 26%.

According to American College of Rheumatology guidelines (8).

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Figure 3. Distribution of synovial fluid leukocyte count (A) and neutrophil percentage (B) in study subjects with prosthetic joint infection, by microorganism type and whether or not antimicrobial agents had been administered.
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standardized and can be automated (31). A major limitation of synovial fluid cell count analysis, however, is that it does not identify the causative microorganism, an essential element in the selection of appropriate antimicrobial therapy. Therefore, cultures are still needed. Nevertheless, synovial fluid cell count can help distinguish true-positive from false-positive results.

Our study has several limitations. First, we did not include subjects with inflammatory joint diseases, which are known to be associated with high synovial fluid leukocyte counts and neutrophil percentage (10). Osteoarthritis is, however, the most common joint disease leading to arthroplasty, and the number of patients with osteoarthritis will continue to increase as the population ages. At the same time, inflammatory diseases are increasingly controlled with medical treatment (32,33). Second, our study included only knee prostheses; it is not known whether the proposed cutoff values are valid for other prosthetic joints. Third, the time between joint aspiration and synovial fluid analysis and the use of heparin for synovial fluid preservation may have influenced cell counts (34). However, synovial fluid specimens were preserved in ethylenediaminetetraacetic acid, which has been shown to yield accurate cell counts for at least 24 hours (31). Most of the specimens were analyzed within 6 hours of joint aspiration. Furthermore, any short delay in synovial fluid analysis should not have systemically biased our data. Treatment of clotted synovial fluid with hyaluronidase might have affected the leukocyte count, but not the neutrophil percentage.

In summary, as the number of patients with prosthetic joints increases, synovial fluid analysis will be used increasingly in the evaluation of prosthetic joint disorders. Our study highlights the clinical utility of synovial fluid cell count evaluation in the diagnosis of prosthetic joint infection. In patients without underlying inflammatory joint disease, a synovial fluid leukocyte differential of >65% neutrophils (or a total leukocyte count of >1.7 x 10^7/μL) is highly predictive of prosthetic knee infection.

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