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The Role of Intraoperative Gram Stain in Revision Total Joint Arthroplasty

Craig J. Della Valle, MD,* David M. Scher, MD,* Yong H. Kim, MD,* Cubyson M. Oxley, BA,* Panna Desai, MD,† Joseph D. Zuckerman, MD,* and Paul E. Di Cesare, MD*

Abstract: The ability to identify intraoperatively patients with an infected prosthesis at the time of a revision procedure assists the surgeon in selecting appropriate management. The results of 413 intraoperative Gram stains were compared with the results of operative cultures, permanent histology, and the surgeon's intraoperative assessment to determine the ability of Gram stains to identify periprosthetic infection. Gram staining correctly identified the presence of infection in 10 of the 68 cases that met study criteria for infection (sensitivity of 14.7%). Four false-positive Gram stains were encountered. Intraoperative Gram stains do not have adequate sensitivity to be helpful in identifying periprosthetic infection and should not be performed on a routine basis. They may be helpful, however, in cases in which gross purulence is encountered to assist in the selection of initial antibiotic therapy. The use of intraoperative Gram staining alone is inadequate for ruling out infection at the time of revision total joint arthroplasty. **Key words:** Gram stain, infection, revision, total hip arthroplasty, total knee arthroplasty.

The most important factor influencing the management of a failed total joint arthroplasty is the differentiation of septic from aseptic failure [1]. Although history, physical examination, and preoperative diagnostic studies may be highly suggestive of infection, no combination of these can conclusively diagnose or rule out the presence of infection in all cases. Thus, the surgeon must often rely on intraoperative diagnostic tests to assist in determining appropriate management.

Many surgeons obtain an intraoperative Gram stain to assist in this decision-making process, despite data suggesting that this test lacks adequate sensitivity to detect periprosthetic infection [2–8]. Intraoperative Gram stains have been used routinely at our institution during revision total joint arthroplasty; the purpose of this study was to determine the ability of this test to detect active infection at the time of revision or resection arthroplasty.

Materials and Methods

Patients and Study Methods

The cases of 413 patients who underwent either a revision arthroplasty (338 patients) or resection arthroplasty (75 patients) between 1990 and 1997 at our institution and had an intraoperative Gram

*From the *Musculoskeletal Research Center, Department of Orthopaedic Surgery, New York University Medical Center–Hospital for Joint Diseases; and the †Department of Pathology, Hospital for Joint Diseases, Orthopaedic Institute, New York, New York.*

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Reprint requests: Paul E. Di Cesare, MD, Musculoskeletal Research Center, Room 1500, Department of Orthopaedic Surgery, New York University Medical Center–Hospital for Joint Diseases, 301 East 17th Street, New York, NY 10003.

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Table 1. Diagnosis at the Time of Initial Arthroplasty

Hip arthroplasty	
Osteoarthrosis	157
Trauma or fracture	48
Osteonecrosis	28
Rheumatoid arthritis	13
Developmental hip dysplasia	8
Prior septic arthritis	2
Lupus arthritis	2
Perthes' disease	2
Knee arthroplasty	
Osteoarthrosis	124
Rheumatoid arthritis	20
Trauma or fracture	5
Osteonecrosis	4

stain were reviewed. The revision cases included 79 patients who underwent reimplantation of a hip or knee prosthesis as part of a 2-stage exchange arthroplasty. The study group consisted of 251 women and 162 men (mean age, 62.9 years; range, 22–91 years); 260 cases involved a total hip arthroplasty and 153 a total knee arthroplasty. The revision procedure was done at a mean of 73.7 months after the primary arthroplasty had been performed (range, 1–319 months). Diagnoses that led to the original arthroplasties are listed in Table 1, and configurations of the arthroplasties at the time of the revision or resection procedure are shown in Table 2.

Periprosthetic infection was defined as those cases meeting 2 of the 3 following criteria: i) permanent histology consistent with acute inflammation; ii) positive intraoperative cultures; or iii) intraoperative appearance of grossly infected tissues (ie, purulence) [1,2]. Intraoperative Gram stains were ordered at the discretion of the attending orthopedic surgeon. Prophylactic perioperative antibiotics were withheld before the collection of intraoperative cultures in all cases.

Permanent histology was considered to be consistent with infection if an average of more than 10 polymorphonuclear cells were visualized in the 5 most cellular-appearing fields under 40× magnification [5,9]. Intraoperative Gram stains were considered positive if bacteria were visualized. In those cases in which bacteria were identified, the morphology of the organism was compared to the results of final cultures to determine the ability of Gram stains to identify the infecting organisms correctly. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were determined [10].

Table 2. Configuration of Arthroplasty at the Time of Revision or Resection

Hip arthroplasty	
Cemented cup and stem	107
Hybrid*	64
Noncemented cup and stem	39
Reverse hybrid†	5
Noncemented hemiarthroplasty	3
Cemented hemiarthroplasty	3
Cup arthroplasty	1
Antibiotic-impregnated cement spacer	38
Knee arthroplasty	
Cemented	110
Noncemented	2
Antibiotic-impregnated cement spacer	41

*Hybrid—cemented stem and noncemented cup.
†Reverse hybrid—noncemented stem and cemented cup.

Gram Stain Procedure

Specimens for Gram staining were thinly and evenly smeared onto 25 × 75 × 1-mm slides (Fisher Scientific, Pittsburgh, PA) that had been cleaned with 95% ethanol; specimens were then fixed by passing the slide over a flame. The slide was flooded with crystal violet for 1 minute, washed with water, and then flooded with iodine solution for 1 minute (Difco Laboratories, Detroit, MI). The slide was decolorized for several seconds, washed in water, and then counterstained with Safranin-O solution for 1 minute. The slide was blotted dry and then examined under 100× magnification using an oil immersion lens. A technician interpreted the slides, and the results were reported to the operating room.

Results

Sixty-eight patients met the study design criteria for infection. The infecting organisms identified for these cases are listed in Table 3. There were a total of 14 positive Gram stains; 10 were considered true positive and 4 false positive. The remaining cases included 341 true negatives and 58 false negatives. The sensitivity of intraoperative Gram stains in detecting infection was 14.7%, with a specificity of

Table 3. Organism Identified in 68 Cases Meeting Study Criteria for Infection

Staphylococcus epidermidis	24
Staphylococcus aureus	14
Enterococcus faecalis	6
Streptococcus viridans	5
Enterobacter cloacae	3
Group B streptococcus	2
Escherichia coli	1
Multiple organisms	1
No organism identified	12

98.8%, negative predictive value of 85.4%, positive predictive value of 71.4%, and accuracy of 84.9%.

Gross purulence was encountered intraoperatively in 6 of the 10 cases in which the Gram stain represented a true positive. The remaining 4 cases all had an intraoperative appearance that was suspicious for infection as well as significant clinical suspicion for infection based on preoperative evaluation.

Nine of the 10 true-positive Gram stains revealed gram-positive cocci; gram-positive organisms grew on all 9 cultures (6 *Staphylococcus aureus*, 2 *Staphylococcus epidermidis*, and 1 *Enterococcus faecalis*). The tenth true-positive Gram stain revealed gram-positive cocci and gram-negative rods; multiple organisms were cultured, including group A streptococcus, *S. aureus*, and *Pseudomonas aeruginosa*.

The 4 false-positive Gram stains all showed few gram-positive cocci; in all these cases, the intraoperative appearance, final cultures, and permanent histology were negative for infection. Two of the patients whose specimens yielded false-positive results underwent primary exchange arthroplasty because the overall clinical picture was consistent with aseptic loosening; the other 2 patients underwent resection arthroplasty followed by delayed reimplantation after the results of permanent histology and final cultures were available and found to be negative.

The charge for doing an intraoperative Gram stain at our institution is $14.50; the total cost of performing this test for these 413 cases was thus $5,988.50, which correctly identified infection in 10 cases (a cost of $598.85 per true-positive result). As previously discussed, however, all of these cases had an intraoperative appearance, preoperative evaluation, or both that were highly suggestive of infection, and thus the patient's treatment was not changed on the basis of any of the true-positive results.

Discussion

The ability to identify intraoperatively those patients with an infected prosthesis at the time of a revision procedure assists the surgeon in selecting appropriate management. Given the limitations of preoperative history and physical examination, serology, hip aspiration, and nuclear medicine studies in identifying the presence or absence of infection, intraoperative diagnosis or confirmation of the surgeon's preoperative assessment is often necessary [1,2]. Intraoperative Gram stains are often performed at the time of revision arthroplasty to assist in this differentiation and have been accepted as an important part of the surgeon's intraoperative assessment [11].

Numerous authors have commented on the ability of intraoperative Gram stains to detect periprosthetic infections. Barrack and Harris [2] reported that the intraoperative Gram stains were negative in the 6 infected hips in their series; Feldman et al. [5] reported that intraoperative Gram stains detected only 2 of 10 infections. Similarly, Athanasou et al. [8] reported that intraoperative Gram stains were positive in 4 of 23 infected arthroplasties, and Kramer et al. [6] reported that 3 of 20 infected hip prostheses had a positive intraoperative Gram stain. In a much larger series of 194 revision arthroplasties in which intraoperative Gram stains were performed, none of the 32 infections were detected [4]. The present study, reporting on 413 revision total joint arthroplasties, is the largest study to date on this topic that we are aware of and finds a similarly low sensitivity that precludes, in our opinion, the routine use of intraoperative Gram stains at the time of revision surgery. In addition, this study reports a nearly 1% rate of false-positive Gram stains, despite numerous precautions to prevent such occurrences, a finding not previously reported, to our knowledge, in the orthopedic literature.

In all false-positive cases, the positive Gram stain was the only indicator of infection; thus, a false-positive Gram stain can be misleading. Two of the 4 patients who had a false-positive intraoperative Gram stain underwent resection arthroplasty followed by reimplantation after the final histology and cultures were found to be negative. These cases emphasize the importance of avoiding the routine use of a test with limited sensitivity because the risks associated with acting on the results of such a test may outweigh its potential benefits.

False-positive Gram stains have been reported in association with procedures such as spinal taps [12], percutaneous amniocenteses [13], and intraoperative specimens collected from the biliary tree [14]; false-positive rates as high as 12% have been reported in these circumstances. Sources for false-positive smears include contaminated alcohol storage baths and Gram-staining reagents [12], technical errors in the preparation and interpretation of Gram-stained slides, and contamination of the field being sampled. Crystal formation in Gram-staining solutions, which may also yield false-positive results, was believed to be responsible for 2 of the false-positive results observed in our study. It has also been our experience that contamination of tissues with methylene blue used intraoperatively

can yield false-positive smears. Contamination of an automated Gram-staining apparatus has also been reported [15], as has contamination of culture collection devices [16].

Intraoperative Gram stains, when positive, did accurately predict the morphology of the infecting organism. This fact indicates that intraoperative Gram stains have a limited role in cases in which grossly infected tissues or purulence is encountered, by assisting with the initial selection of antibiotics if no organism has been identified by preoperative studies.

In an era when increasing emphasis is placed on both cost containment and cost-effectiveness, the judicious use of adjunctive testing must be closely examined. In the present analysis, the cost of performing intraoperative Gram stains added a total of approximately $6,000 to the cost of managing these 413 patients. Although the total cost of performing the test itself is small when compared with the total cost of a revision total joint arthroplasty, the utility of the test was low. Specifically, the Gram stain was not helpful in establishing the presence of sepsis in any of these cases because all of them had an intraoperative appearance, preoperative evaluation, or both that were highly suggestive of infection. Conversely, 2 patients did not undergo primary exchange arthroplasty on the basis of a false-positive Gram stain, which added significantly to the cost of managing these patients (ie, because of an extended hospitalization and a second operative procedure).

The present study is limited by its retrospective nature and by the fact that the decision to order a Gram stain was in each case made intraoperatively by the surgeon rather than universally mandated as in a prospective series. This bias, however, would tend to favor Gram staining during cases in which there was a higher suspicion of infection; this bias would tend only to increase the sensitivity of the test, which is already below a level that was found to be useful. The large sample size of the study and the significant percentage of infected cases (16.5%) may have compensated for any bias introduced into the study by its retrospective nature and nonconsecutive design.

Conclusion

Intraoperative Gram stains do not have adequate sensitivity to detect periprosthetic infection and should not be ordered on a routine basis because more sensitive modalities, such as intraoperative frozen section, are available [5,9]. They may be helpful, however, in cases in which grossly infected-appearing tissues or purulence is encountered to assist in the selection of initial antibiotic therapy; in such cases, the Gram stains, when positive, were able to identify accurately the morphology of the infecting organism. The use of intraoperative Gram staining alone is inadequate for ruling out infection at the time of revision total joint arthroplasty.

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