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The Value of Intraoperative Gram Stain in Revision Total Knee Arthroplasty

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Background: The accurate preoperative diagnosis of infection is an essential component of decision-making prior to revision total knee arthroplasty. When preoperative modalities used to detect infection reveal equivocal findings, the surgeon may rely on intraoperative testing. While intraoperative Gram stains are routinely performed during revision total knee arthroplasty, their value remains unclear.

Methods: We retrospectively reviewed the records on 945 revision total knee arthroplasties performed at three university institutions to which patients were referred for total joint arthroplasty; the results of an intraoperative Gram stain were available for review in 921 cases (97.5%). Of these knees, 247 were classified as infected on the basis of (1) the presence of the same organism in two cultures; (2) growth, on solid media, of an organism as well as other objective evidence of infection; (3) histologic evidence of acute inflammation; (4) gross purulence; and/or (5) an actively draining sinus. We reviewed the results of preoperative laboratory studies, which included measurements of the erythrocyte sedimentation rate, C-reactive protein values, and white blood-cell count in 90%, 76%, and 98% of cases, respectively. Preoperative aspiration to obtain a specimen for culture and a cell count was performed routinely at one center and selectively at the other two centers, and the results were available for review in 439 (48%) of the 921 cases.

Results: Intraoperative Gram staining was found to have a sensitivity of 27% and a specificity of 99.9%. The positive and negative predictive values were 98.5% and 79%, respectively. The test accuracy was 80%. Patients with a true-positive Gram stain had a significantly higher preoperative white blood-cell count, C-reactive protein level, and nucleated cell count in the aspirate when compared with patients with a false-negative Gram stain (p < 0.001). In no case did the results of the intraoperative Gram stain alter treatment.

Conclusions: The intraoperative Gram stain was found to have poor sensitivity and a poor negative predictive value, and its results did not alter the treatment of any patient undergoing revision total knee arthroplasty because of a suspected infection. These data do not support the routine use of intraoperative Gram staining in revision total knee arthroplasty; instead, they suggest a much more limited role for this test.

Level of Evidence: Diagnostic Level I. See Instructions to Authors for a complete description of levels of evidence.

Total knee arthroplasty is a successful and effective surgical treatment for arthritis, with reported survivorship and patient satisfaction rates of >90% at ten to fifteen years[1,2]. A knee arthroplasty that does fail, however, poses a management dilemma. Paramount to choosing an appropriate treatment strategy is the correct identification of the cause of failure. While noninfectious etiologies such as loosening, instability, and malalignment are responsible for the majority of total knee revisions, infection continues to be the reason for a substantial percentage of revisions and has been reported to be

TABLE I Preoperative Testing Performed on Patients with an Intraoperative Gram Stain				
	No. of Patients with Test			
Result of Gram Stain	White Blood-Cell Count	Erythrocyte Sedimentation Rate	C-Reactive Protein	Aspiration
True-positive (67 patients)	67	66	58	47
False-negative (180 patients)	177	175	157	142
True-negative (673 patients)	658	589	481	238
False-positive (1 patient)	1	1	1	0
Total (921 patients)	903 (98%)	831 (90%)	697 (76%)	439 (48%)

the single most common cause of early failure[3-5]. Two-stage exchange arthroplasty is generally considered the preferred method of treatment of periprosthetic joint infection[6-9].

A preoperative evaluation that incorrectly identifies the presence or absence of infection can lead to either inappropriate surgical intervention or a delay in appropriate treatment. Currently, however, there is no gold standard for the diagnosis of an infection at the site of a total knee arthroplasty. An equivocal result of a preoperative workup may, therefore, require the surgeon to depend on intraoperative tests to determine that infection is absent[10]. While the efficacy of frozen-section analysis has been established by several authors[11-13], the value of an intraoperative Gram stain remains unclear. Although the test is routine at many centers, its sensitivity has been consistently reported to be low and previous studies have been hampered by small sample sizes or by the authors combining the results of both hip and knee revision surgery[11-21].

This study was designed to determine the sensitivity, specificity, accuracy, and positive and negative predictive values of intraoperative Gram stains in a large cohort of patients undergoing revision total knee arthroplasty. We also undertook a review of the preoperative and intraoperative findings to determine whether there were instances in which the intraoperative Gram stain may have provided valuable information to the surgeon. In doing so, we hoped to identify the role that an intraoperative Gram stain plays in determining the correct course of treatment.

Materials and Methods

The data for this study were prospectively gathered at three university-affiliated institutions over a six-year period and entered into an institutional review board-approved, multi-institution database of information on all revision total knee arthroplasties. This database was queried retrospectively for information relevant to the study question. Routine preoperative testing included measurement of the erythrocyte sedimentation rate, C-reactive protein level, and white blood-cell count (Table I). Preoperative aspiration was performed routinely at one center, and it was carried out selectively at the other two institutions when infection was suspected on the basis of clinical or radiographic findings. Aspirates were sent for aerobic and anaerobic culture and, when sufficient fluid was available, for a cell count and differential. Aspiration results were available for

439 patients (48%). An intraoperative culture of synovial fluid was performed in all cases, and additional tissue was sent for culture in all but three cases. The results of the preoperative and intraoperative cultures and the medium on which the bacteria were identified (solid, or enhanced broth) were documented. Clinical follow-up notes were reviewed for evidence of infection.

Knees were classified as infected when three of the five following criteria described by Leone and Hanssen[22] were met: (1) the presence of the same organism in two cultures, (2) growth of an organism on solid media as well as other objective evidence of infection such as elevated levels of inflammatory markers in the absence of systemic inflammatory disease or an elevated cell count and percentage of polymorphonuclear leukocytes in aspirated joint fluid, (3) histologic evidence of acute inflammation, (4) gross purulence at the time of surgery, or (5) an actively draining sinus. Threshold values for evidence of infection based on results of blood tests and synovial fluid analysis were derived from the published literature and included an erythrocyte sedimentation rate of >30 mm/hr, a C-reactive protein level of >10 mg/L, a synovial fluid nucleated cell count of >1700 cells/μL, a white blood-cell count of >11.0 × 10^9/L, and a synovial fluid leukocyte differential of >65% polymorphonuclear leukocytes[15,23]. A normal result of a preoperative workup was defined as one in which all of its components were within these defined normal limits.

Statistical Analysis

The Mann-Whitney one-tailed t test with Gaussian approximation, and calculations of sensitivity, specificity, accuracy, and positive and negative predictive values, were performed with use of GraphPad Prism, version 5.01 for Windows (GraphPad Software, San Diego, California). Descriptive analysis was performed with use of univariate statistics for the continuous variables and frequency distribution for the categorical variables. Results for the continuous variables are reported as means and range distributions. Gaussian distribution was evaluated to determine if there was a normal distribution of the data. Chi-square analysis was carried out to determine the sensitivity, specificity, accuracy, and positive and negative predictive values. With use of one-tailed Mann-Whitney statistics, the means and standard deviations for the continuous variables, including the erythrocyte sedimentation rate, C-reactive protein level, white blood-cell count, cell count in the aspirate, and percentage of

TABLE II 2 × 2 Table for Results of Gram Staining for Diagnosis of Infection During Revision Total Knee Arthroplasty		
	Infection Confirmed*	
Gram Stain	Yes	No
Positive	67 true-positive	1 false-positive
Negative	180 false-negative	673 true-negative

*True-positive = infection present and Gram stain positive, false-positive = infection absent and Gram stain positive, false-negative = infection present and Gram stain negative, and true-negative = infection absent and Gram stain negative. Sensitivity = true-positive/(true-positive + false-negative) or true-positive/total with disease = 0.27 (95% confidence interval, 0.22 to 0.33). Specificity = true-negative/(false-positive + true-negative) or true-negative/total without disease = 0.999 (95% confidence interval, 0.99 to 1.00). Positive predictive value = true-positive/(true-positive + false-positive) or true-positive/all with positive test = 0.985 (95% confidence interval, 0.92 to 1.00). Negative predictive value = true-negative/(false-negative + true-negative) or true-negative/all with negative test = 0.79 (95% confidence interval, 0.76 to 0.82).

polymorphonuclear leukocytes in the aspirate, were compared between the patients with a true-positive result and the ones with a false-negative result. The Mann-Whitney statistic is a non-parametric test and was used for this analysis as the data were collected from independent samples and were not normally distributed. The results of these comparisons are reported as p values.

Source of Funding

Results

The records on 945 consecutive revision total knee arthroplasties performed over a six-year period were reviewed; the results of an intraoperative Gram stain were available for review in 921 (97.5%) of these cases. Two hundred and forty-seven knees were classified as infected, and all had an intraoperative Gram stain available for review. Of the 698 knees determined not to be infected, 674 had a Gram stain available for review; the Gram stain was reported to be negative in 673 of these cases and positive in one. In the positive case, all other tests (white blood-cell count and measurements of the erythrocyte sedimentation rate and level of C-reactive protein) demonstrated normal results and an intraoperative frozen section showed no acute inflammation. A revision was performed with no subsequent evidence of infection, so the Gram stain was classified as false-positive. Intraoperative Gram staining was found to have a sensitivity of 27% and a specificity of 99.9%. The positive and negative predictive values were 98.5% and 79%, respectively (Table II). Intraoperative Gram staining did not influence treatment in any case.

There were sixty-seven true-positive and 180 false-negative Gram stains (Table III). The white blood-cell count, C-reactive protein level, and aspirate cell count were significantly higher in the group of infected knees with a true-positive Gram stain than they were in the group with a false-negative Gram stain (p < 0.001). The erythrocyte sedimentation rate and differential cell count (percentage of polymorphonuclear leukocytes) in the joint fluid aspirate did not differ significantly between the two groups (p = 0.3 and p = 0.4, respectively) (Table III). The erythrocyte sedimentation rate, C-reactive protein level, aspirate cell count, and percentage of polymorphonuclear leukocytes in the aspirate were all significantly higher in the infected knees with a negative Gram stain (false-negative cases) than they were in the uninfected knees with a negative Gram stain (true-negative cases) (p < 0.01); this finding was consistent with the results in a large body of literature[7,13,15,24]. Of the sixty-seven patients with a true-positive Gram stain, only one had normal results of the preoperative workup (white blood-cell count, erythrocyte sedimentation rate, and C-reactive protein level). A preoperative aspiration had not been performed in that patient, and purulent fluid was encountered. On the basis of the intraoperative appearance of the tissue, a frozen-section analysis was performed and it showed acute inflammation (>10 polymorphonuclear leuko-

TABLE III Comparison of Preoperative Laboratory Results for Infected Knees with Positive and Negative Intraoperative Gram Stains			
Parameter	True-Positive* (N = 67)	False-Negative* (N = 180)	P Value
White blood-cell count ($\times 10^9$/L)	11.8 ± 5.159 (4.4-35.6)	8.6 ± 3.802 (3.4-38.9)	<0.001
Erythrocyte sedimentation rate (mm/hr)	81.15 ± 32.66 (4-141)	72.7 ± 30.12 (1-140)	0.3
C-reactive protein (mg/L)	22.8 ± 20.28 (0.5-100)	15.23 ± 66.08 (0.5-149)	<0.001
Aspirate nucleated cell count (cells/μL)	60,000 ± 120,000 (175-585,000)	27,600 ± 100,000 (330-850,000)	0.001
Aspirate polymorphonuclear leukocytes (%)	86.27 ± 12.97 (17-100)	85.7 ± 16.38 (4-99)	0.4

*The values are given as the mean and standard deviation with the range in parentheses.

TABLE IV Results of Intraoperative Gram Staining for the Detection of Periprosthetic Infection as Reported in the Literature

Authors	Year	No. of Cases	Site	Sensitivity (%)	Specificity (%)
Athanasou et al.[11]	1995	106	Hip/knee	18	100
Atkins et al.[14]	1998	297	Hip/knee	12	98
Barrack et al.[15]	1997	69	Knee	10	100
Bauer et al.[13]	2006	168	Hip/knee	22 and 35*	100
Chimento et al.[16]	1996	194	Hip/knee	0	0
Della Valle et al.[17]	1999	413	Hip/knee	14.7	98.8
Feldman et al.[12]	1995	33	Hip/knee	22	100
Ko et al.[18]	2005	40	Hip/knee	0	0
Kraemer et al.[19]	1993	144	Hip	23	100
Pandey et al.[20]	1999	602	Hip	21.5	100
Spangehl et al.[21]	1999	202	Hip	19	98

*Tissue and fluid, respectively.

cytes per high-power field). A two-stage exchange arthroplasty was performed.

Discussion

Described by Hans Christian Gram in 1884, the Gram stain exploits biochemical differences between bacterial cell walls to broadly classify many bacteria as either gram-positive or gram-negative[25]. This categorization is based on a number of morphological characteristics of the bacterium, including the relative thickness of the bacterial peptidoglycan layer and the presence or absence of an outer membrane. We are not aware of any available data concerning the average bacterial load seen within the tissues of an infection at the site of a total knee arthroplasty. Periprosthetic infection often occurs with a low organism burden in the synovial fluid. This is influenced by the formation of biofilms, which have a higher organism burden, and this may in part explain the variable sensitivity of Gram stains reported for different organisms[26,27]. The results of the present study indicate that an intraoperative Gram stain is more likely to be positive in the setting of a more fulminant periprosthetic knee infection, a hypothesis supported by the fact that the white blood-cell count, C-reactive protein level, and aspirate cell count were significantly higher in cases with a true-positive Gram stain than they were in those with a false-negative stain.

A Gram stain is commonly performed on operatively retrieved specimens as a means of screening for the presence of infection[24,28], but this practice appears to be of questionable value. Previous studies of relatively small cohorts have shown Gram stains to have poor sensitivity, which has been as low as 0% in some reports (Table IV). Indeed, previous authors who reviewed a mixed cohort of hip and knee revisions questioned the value of an intraoperative Gram stain and suggested that the test should not be ordered on a routine basis[17]. The sensitivity of an intraoperative Gram stain was also low (27%) in our series of 921 revision total knee arthroplasties.

Preoperative planning of the treatment of a failed total knee arthroplasty depends in part on the results of a preoperative workup, in which identification of infection is important. The intraoperative use of a Gram stain had little or no diagnostic role for the patients with positive results of the preoperative workup for infection in our series. The sensitivity of the intraoperative Gram stain also was too low to be considered reliable for the patients with equivocal results of the preoperative workup. In addition, the Gram stain proved to be of no value for the patients with completely normal results of the preoperative workup since no true-positive cases were identified within this group and there was one false-positive case.

There is a potential for substantial variability in the interpretation of the Gram stain by the laboratory personnel performing the test. There is the potential for error in both the staining process and the interpretation of the slides, problems that may have contributed to the prevalence of false-negative results observed in this study. False-positive cases were rare in this series (only one case), but less experienced technicians could overinterpret Gram-stain results, leading to more false-positive findings. Specimen contamination can also result in a false-positive finding. Previous investigators have reported that intraoperative Gram staining has a very high specificity for a positive result[17,21]. Our study confirms this finding. Of the sixty-seven patients with a true-positive Gram stain, only one had normal findings on the preoperative workup, which was incomplete. The patient was an eighty-year-old man who was noted to have purulent joint fluid at the time of surgery; a preoperative joint aspiration had not been performed. An intraoperative frozen-section analysis was performed, and re-implantation was aborted because of the gross purulence. Therefore, the intraoperative Gram stain did not alter the treatment of any patient.

The study had some weaknesses. First, as a result of its retrospective design, there may have been some variability in data collection. Second, because it was a multi-institutional study, it is possible that the protocols for the workup and management differed among the patients. In fact, this was the case with respect to aspiration of the joint. All knees scheduled to undergo revision arthroplasty were aspirated routinely in one institution and only selectively in the others. This resulted in a lack of availability of aspiration data for some patients. Also, because of differences in the workup of these patients, serological tests such as measurement of the C-reactive protein level were not performed for all patients. Such variability in the management of these patients presents the possibility of bias; specifically, it is possible that the relative value of diagnostic tests other than the Gram stain may have been incorrectly exaggerated or diminished. This study, however, was not designed or intended to establish the value of either measurement of the C-reactive protein level or preoperative aspiration in the diagnosis of periprosthetic infection.

A complete preoperative workup for periprosthetic infection in a patient with a failed total knee arthroplasty includes serological testing, synovial fluid analysis, and radiographic imaging. We consider preoperative aspiration to be particularly useful. The accuracy of preoperative aspiration for the diagnosis of infection prior to revision of a failed total knee arthroplasty has been reported to be very high, approaching 100% in some series[13,29] but generally ranging from 75% to 80% if the patient is not being treated with antibiotics[15]. In our series, in which strict criteria were employed for the definition of periprosthetic infection, intraoperative Gram staining played no role in the diagnosis of infection in patients who had had a full preoperative workup and the selective use of intraoperative frozen-section analysis. We suggest that the practice of routinely performing a Gram stain at the time of revision total knee arthroplasty may safely be abandoned. ∎

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References

1. Colizza WA, Insall JN, Scuderi GR. The posterior stabilized total knee prosthesis. Assessment of polyethylene damage and osteolysis after a ten-year-minimum follow-up. J Bone Joint Surg Am. 1995;77:1713-20.

2. Font-Rodriguez DE, Scuderi GR, Insall JN. Survivorship of cemented total knee arthroplasty. Clin Orthop Relat Res. 1997;345:79-86.

3. Sharkey PF, Hozack WJ, Rothman RH, Shastri S, Jacoby SM. Why are total knee arthroplasties failing today? Clin Orthop Relat Res. 2002;404:7-13.

4. Fehring TK, Odum S, Griffin WL, Mason JB, Nadaud M. Early failures in total knee arthroplasty. Clin Orthop Relat Res. 2001;392:315-8.

5. Weir DJ, Moran CG, Pinder IM. Kinematic condylar total knee arthroplasty. 14-year survivorship analysis of 208 consecutive cases. J Bone Joint Surg Br. 1996;78:907-11.

6. Windsor RE, Insall JN, Urs WK, Miller DV, Brause BD. Two-stage reimplantation for the salvage of total knee arthroplasty complicated by infection. Further follow-up and refinement of indications. J Bone Joint Surg Am. 1990;72:272-8.

7. Callaghan JJ, Salvati EA, Brause BD, Rimnac CM, Wright TM. Reimplantation for salvage of the infected hip: rationale for the use of gentamicin-impregnated cement and beads. Hip. 1985:65-94.

8. Walenkamp GH, Vree TB, van Rens TJ. Gentamicin-PMMA beads. Pharmacokinetic and nephrotoxicological study. Clin Orthop Relat Res. 1986;205:171-83.

9. Antti-Poika I, Josefsson G, Konttinen Y, Lidgren L, Santavirta S, Sanzén L. Hip arthroplasty infection. Current concepts. Acta Orthop Scand. 1990;61:163-9.

10. Levitsky KA, Hozack WJ, Balderston RA, Rothman RH, Gluckman SJ, Maslack MM, Booth RE Jr. Evaluation of the painful prosthetic joint. Relative value of bone scan, sedimentation rate, and joint aspiration. J Arthroplasty. 1991;6:237-44.

11. Athanasou NA, Pandey R, de Steiger R, Crook D, Smith PM. Diagnosis of infection by frozen section during revision arthroplasty. J Bone Joint Surg Br. 1995;77:28-33.

12. Feldman DS, Lonner JH, Desai P, Zuckerman JD. The role of intraoperative frozen sections in revision total joint arthroplasty. J Bone Joint Surg Am. 1995;77:1807-13.

13. Bauer TW, Parvizi J, Kobayashi N, Krebs V. Diagnosis of periprosthetic infection. J Bone Joint Surg Am. 2006;88:869-82.

14. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, McLardy-Smith P, Berendt AR. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. J Clin Microbiol. 1998;36:2932-9.

15. Barrack RL, Jennings RW, Wolfe MW, Bertot AJ. The value of preoperative aspiration before total knee revision. Clin Orthop Relat Res. 1997;345:8-16.

16. Chimento GF, Finger S, Barrack RL. Gram stain detection of infection during revision arthroplasty. J Bone Joint Surg Br. 1996;78:838-9.

17. Della Valle CJ, Scher DM, Kim YH, Oxley CM, Desai P, Zuckerman JD, Di Cesare PE. The role of intraoperative Gram stain in revision total joint arthroplasty. J Arthroplasty. 1999;14:500-4.

18. Ko PS, Ip D, Chow KP, Cheung F, Lee OB, Lam JJ. The role of intraoperative frozen section in decision making in revision hip and knee arthroplasties in a local community hospital. J Arthroplasty. 2005;20:189-95.

19. Kraemer WJ, Saplys R, Waddell JP, Morton J. Bone scan, gallium scan, and hip aspiration in the diagnosis of infected total hip arthroplasty. J Arthroplasty. 1993;8:611-6.

20. Pandey R, Drakoulakis E, Athanasou NA. An assessment of the histological criteria used to diagnose infection in hip revision arthroplasty tissues. J Clin Pathol. 1999;52:118-23.

21. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am. 1999;81:672-83.

22. Leone JM, Hanssen AD. Management of infection at the site of a total knee arthroplasty. J Bone Joint Surg Am. 2005;87:2335-48.

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23. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med. 2004;117:556-62.

24. Spangehl MJ, Younger AS, Masri BA, Duncan CP. Diagnosis of infection following total hip arthroplasty. Instr Course Lect. 1998;47:285-95.

25. Gram C. The differential staining of Schizomycetes in tissue sections and in dried preparations. Fortschritte der Medizin. 1884;2:185-9.

26. Marker D, Seyler TM, Delanois RE, Plate JF, Mont MA. The sensitivity of Gram stains from multiple sites in patients with infected total hip arthroplasty. Presented as a poster exhibit at the Annual Meeting of the American Academy of Orthopaedic Surgeons; 2007 Feb 14-18; San Diego, CA. Poster no. 048.

27. Tunney MM, Ramage G, Patrick S, Nixon JR, Murphy PG, Gorman SP. Antimicrobial susceptibility of bacteria isolated from orthopedic implants following revision hip surgery. Antimicrob Agents Chemother. 1998;42:3002-5.

28. Moyad TF, Thornhill T, Estok D. Evaluation and management of the infected total hip and knee. Orthopedics. 2008;31:581-8; quiz 589-90.

29. Duff GP, Lachiewicz PF, Kelley SS. Aspiration of the knee joint before revision arthroplasty. Clin Orthop Relat Res. 1996;331:132-9.