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Microbiologic Diagnosis of Prosthetic Shoulder Infection by Use of Implant Sonication▽

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We recently described a sonication technique for the diagnosis of prosthetic knee and hip infections. We compared periprosthetic tissue culture to implant sonication followed by sonicate fluid culture for the diagnosis of prosthetic shoulder infection. One hundred thirty-six patients undergoing arthroplasty revision or resection were studied; 33 had definite prosthetic shoulder infections and 2 had probable prosthetic shoulder infections. Sonicate fluid culture was more sensitive than periprosthetic tissue culture for the detection of definite prosthetic shoulder infection (66.7 and 54.5%, respectively; $P = 0.046$). The specificities were similar (98.0% and 95.1%, respectively; $P = 0.26$). *Propionibacterium acnes* was the commonest species detected among culture-positive definite prosthetic shoulder infection cases by periprosthetic tissue culture (38.9%) and sonicate fluid culture (40.9%). All subjects from whom *P. acnes* was isolated from sonicate fluid were male. We conclude that sonicate fluid culture is useful for the diagnosis of prosthetic shoulder infection.

The frequency of shoulder replacement surgery is increasing (1). The incidence of prosthetic shoulder infection varies from 0.4 to 15.4% (6, 7). When an infection is present, the infection requires unique medical and surgical management, rendering an accurate diagnosis critical. However, since patients with prosthetic shoulder infection often present with stiffness and/or pain alone (7), the achievement of an accurate diagnosis is challenging.

Periprosthetic tissue has been the specimen cultured for the microbiologic diagnosis of prosthetic shoulder infection. Specificity is an issue, as microorganisms (e.g., *Propionibacterium* and *Staphylococcus* spp.) can be contaminants, and the number of microorganisms in tissue is small. As a result, it has been suggested that multiple samples be obtained; for prosthetic hips and knees, it is recommended that five or six periprosthetic tissue specimens be cultured (2). No such data are available for shoulder implants.

We recently clinically validated a sonication technique that is used to sample biofilm bacteria on the surface of removed hip and knee implants placed in solid containers. We demonstrated that the culture of samples obtained by sonication of the implant was more sensitive than the culture of periprosthetic tissue for the diagnosis of prosthetic hip and knee infections (22). The poor sensitivity of the latter likely relates to the presence of bacteria in biofilms on the prosthesis surface, a site not well sampled when periprosthetic tissue samples for culture are obtained. No data on the accuracy of sonication for the diagnosis of prosthetic shoulder infection are available.

The proportion of patients with shoulder infections due to *Propionibacterium acnes* is significantly greater than the proportion of patients with lower limb infections due to *P. acnes* (12). Sperling et al. reported that *Propionibacterium* spp. account for 16% of prosthetic shoulder infections (16). Franta et al. reported that among 31/282 patients (11%) with unsatisfactory shoulder arthroplasties, positive intraoperative cultures were found in 23 at the time of revision surgery, with the most common organisms isolated being coagulase-negative *Staphylococcus* spp., followed by *P. acnes* (11). Cheung et al. reported the results of reimplantation of glenoid components following removal and allogeneic bone grafting in seven patients; specimens from two patients demonstrated the growth of *P. acnes* (5). These two patients had continuing pain and radiographic evidence of glenoid component loosening and subsequently underwent repeat revision surgery, whereas the remaining patients did well and did not require repeat revision surgery (5), suggesting a role for *P. acnes* in pain and component loosening. Accordingly, the accurate detection of a *Propionibacterium* spp. is paramount in the diagnosis of prosthetic shoulder infection.

The purpose of the present study was to compare implant sonication to periprosthetic tissue culture for the diagnosis of prosthetic shoulder infection. We also evaluated immunofluorescence microscopy and PCR analysis of sonicate fluid to detect prosthetic shoulder infection caused by the two most frequently associated microorganisms. Finally, we compared patient characteristics associated with *Propionibacterium* prosthetic shoulder infection versus those associated with non-*Propionibacterium* prosthetic shoulder infection.

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MATERIALS AND METHODS

Study population. Patients who underwent revision or resection of shoulder prostheses at our institution between August 2004 and November 2008 were studied. Patients were excluded if less than two periprosthetic tissue specimens were submitted for culture, sonicate fluid was not archived, or a partial revision was performed.

Patient classification. Patients were classified as having definite prosthetic shoulder infection if at least one of the following was present: (i) visible purulence surrounding the prosthesis, (ii) acute inflammation on histopathologic examination of permanent tissue sections, or (iii) a sinus tract communicating with the prosthesis. Patients were classified as having probable prosthetic shoulder infection if they did not meet these criteria but the same organism was isolated from at least two periprosthetic tissues and in significant quantity (see the cutoff values below) from the sonicate fluid. Aseptic failure was defined as a failure that did not meet these criteria.

Specimen collection. Intraoperative tissue samples with the most obvious inflammatory changes were collected for histopathology and conventional microbiologic culture. Explanted prostheses were placed in an autoclaved 1-liter polypropylene widemouthed container (Nalgene, Lima, OH) and were cultured within 6 h.

Periprosthetic tissue culture. Periprosthetic tissue specimens were homogenized in 3 ml of brain heart infusion broth for 1 min, and the homogenate was inoculated in aliquots of 0.5 ml onto aerobic and anaerobic sheep blood agar plates (BD Diagnostic Systems), which were incubated at 35 to 37°C in 5 to 7% carbon dioxide aerobically and anaerobically for 2 to 4 days and 7 days, respectively. Turbid thioglycolate broth was subcultured. Periprosthetic tissue culture positivity was defined as isolation of the same organism from two or more tissue specimens.

Sonicate fluid culture. Sterile Ringer's solution (400 ml) was added to each container, and the container was vortexed and sonicated as described previously (22). For the first 45 subjects studied (until 14 December 2005), 0.5 ml of sonicate fluid was directly plated onto aerobic and anaerobic sheep blood agar plates, which were incubated at 35 to 37°C in 5 to 7% CO_2 aerobically and anaerobically for 5 and 7 days, respectively. For the last 91 subjects studied (after 14 December 2005), a 100-fold concentration step and an extended period of anaerobic incubation were added. Sonicate fluid was centrifuged at 3,150 × *g* for 5 min in conical centrifuge tubes. The supernatant was aspirated; and 0.1 ml of sediment was placed onto aerobic and anaerobic sheep blood agar plates, which were incubated at 35 to 37°C in 5 to 7% CO_2 aerobically and anaerobically for 2 to 4 and 14 days, respectively. The microorganisms were enumerated and identified by routine microbiologic techniques. The criteria used to interpret sonicate fluid culture positivity were as follows. A cutoff value of at least 5 CFU per plate was applied to the first 45 subjects studied. A cutoff value of at least 20 CFU per plate was applied to the 91 subjects enrolled after 14 December 2005. The higher cutoff was used due to the addition of a concentration step to the sonicate fluid culture procedure, which yielded higher numbers of microorganisms. Concentrated sonicate fluid from all subjects was frozen in 1-ml aliquots at −70 to −80°C for subsequent immunofluorescence microscopy and PCR analysis.

Immunofluorescence microscopy on sonicate fluid for detection of *Propionibacterium* and *Staphylococcus* spp. One sonicate fluid aliquot (1 ml) from patients with definite prosthetic shoulder and aseptic failure was thawed, centrifuged (5,000 × *g*, 10 min), the supernatant was removed, and slides were prepared from a 100-μl pellet. Ten microliters was dispensed in duplicate onto wells of two glass slides. The slides were air dried and then fixed in 100% methanol for 10 min. The slides were stored at −70 to −80°C. In this study, two antibodies were utilized. These were an anti-*Propionibacterium* sp. mouse monoclonal antibody (undiluted hybridoma-conditioned media) (23), which detected a collection of *P. acnes* and *Propionibacterium avidum* isolates obtained from subjects with biofilm-associated infections, and an anti-*Staphylococcus* sp. mouse monoclonal antibody (QED Bioscience Inc., San Diego, CA), generated with *Staphylococcus aureus*, which detected *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus caprae*/*Staphylococcus capitis*, *S. aureus*, *Staphylococcus lugdunensis*, *Staphylococcus carnosus*, *Staphylococcus simulans*, and *Staphylococcus hominis* isolates from subjects with biofilm-associated infections.

The slides were removed from the freezer and equilibrated to room temperature. A total volume of 20 μl of anti-*Propionibacterium* monoclonal antibody was applied to the wells of two different slides (one of which acted as a negative control for the anti-*Staphylococcus* antibody), and 20 μl of anti-*Staphylococcus* monoclonal antibody was similarly applied to the wells of two other slides (one of which acted as a negative control for the anti-*Propionibacterium* antibody). The slides were incubated at 35 to 37°C in a humidified chamber for 30 min. After incubation, the slides were rinsed with phosphate-buffered saline and allowed to dry; 20 μl of goat anti-mouse IgG secondary antibody conjugated to fluorescein isothiocyanate (Sigma-Aldrich, St. Louis, MO) was applied to each well. The slides were again incubated at 35 to 37°C in a humidified chamber for 30 min and then rinsed and dried. The slides were mounted with glycerol–phosphate-buffered saline mounting medium and two-by-two glass coverslips and read under oil immersion on a fluorescent microscope. Control slides, made with *P. acnes* and *S. aureus*, were included with each run.

PCR for detection of *P. acnes* and *Staphylococcus* spp. in sonicate fluid. One sonicate fluid aliquot (1 ml) from patients with definite prosthetic shoulder and aseptic failure was thawed and centrifuged (5,000 × *g*, 10 min). Genomic DNA was extracted from a 100-μl pellet with a QIAamp DNA minikit (Qiagen, Valencia, CA). A staphylococcal rapid cycle real-time assay targeting *tuf* was used (15). The assay uses two fluorescence resonance energy transfer probe sets that hybridize internally to the primer binding sites of *tuf*, allowing the differentiation of *S. aureus* from coagulase-negative *Staphylococcus* spp. Oligonucleotide primers and probes were obtained from TIB Molbiol (Adelphia, NJ). The PCR mixture consisted of 2 μl LightCycler FastStart DNA Master HybProbe mixture (Roche Applied Science, Indianapolis, IN), 3 mM $MgCl_2$, 1 μM each primer, and 0.2 μM each probe. Two microliters of template DNA was added to 18 μl of the PCR mixture. The cycling parameters consisted of a 10-min, 95°C preincubation, followed by 45 cycles of 95°C for 10 s, 61°C for 8 s, and 72°C for 22 s. Fluorescent resonance energy transfer signals were acquired after the primer annealing phase. This was followed by a postamplification melting curve analysis. DNAs from an *S. aureus* isolate and an *S. epidermidis* isolate in sonicate fluid from an uninfected prosthesis were used as positive controls. Genomic DNA extracted from the sonicate fluid from an uninfected prosthesis and master mix were used as negative controls. The analytical limit of detection of the assay, determined by testing two *S. epidermidis* isolates spiked into sonicate fluid from an uninfected prosthesis, was 2 colonies per PCR.

A *P. acnes* rapid-cycle real-time LightCycler PCR targeting the 16S rRNA gene of *P. acnes* was used. The 16S rRNA gene primers, PArA-1 (5′-AAGCG TGAGTGACGGTAATGGGTA-3′) and PArA-2 (5′-CCACCATAACGTGCT GGCAACAGT-3′), amplify a region of the *P. acnes* 16S rRNA gene. The assay used Universal Probe 73 (5′-GCTGAGGA-3′) from the Universal ProbeLibrary (Roche Applied Science) that hybridizes internally to the primer binding sites. Oligonucleotide primers were obtained from Integrated DNA Technologies, Inc. (Coralville, IA), and the Universal Probe was obtained from Roche Applied Science. The PCR mixture consisted of 2 μl LightCycler FastStart TaqMan Master mixture (Roche Applied Science), 2.5 mM $MgCl_2$, 0.4 μM each primer, and 0.1 μM probe. Two microliters of template DNA was added to 18 μl of the PCR mixture for a final reaction volume of 20 μl. The cycling parameters consisted of a 10 min, 95°C preincubation, followed by 35 cycles of 95°C for 10 s, 55°C for 8 s, and 72°C for 22 s. Probe fluorescent signals were acquired after the elongation phase. This was followed by a postamplification melting curve analysis. Bacterial DNA from a *P. acnes* isolate spiked into sonicate fluid from an uninfected prosthesis was used as a positive control. Genomic DNA extracted from the sonicate fluid from an uninfected prosthesis and the master mixture alone were used as negative controls. The analytical limit of detection of the assay, determined by testing *P. acnes* spiked into sonicate fluid from an uninfected prosthesis, was equivalent to 0.2 colonies per PCR.

Statistical analysis. The characteristics of patients with aseptic failure and those with definite prosthetic shoulder infection were compared and the characteristics of patients with definite *Propionibacterium* infections and those with non-*Propionibacterium* prosthetic shoulder infections were compared by using the Wilcoxon rank-sum test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. The sensitivities and specificities of the methods studied to detect prosthetic shoulder infection were compared by using McNemar's test, a test of paired proportions. A *P* value of less than 0.05 (for a two-sided test) was considered to indicate statistical significance.

RESULTS

One hundred fifty-seven patients were studied. Fifteen patients were excluded from further analysis due to submission of less than two periprosthetic tissue specimens for culture, one

TABLE 1. Characteristics of study patients

Characteristic	Probable prosthetic shoulder infection (n = 2)	Aseptic failure (n = 101)	Definite prosthetic shoulder infection (n = 33)	P[a]
Demographics				
Median (range) age (yr)	57 (39–75)	67 (40–87)	60 (44–81)	0.19
No. (%) of subjects of female gender	0 (0)	64 (63.4)	11 (33.3)	0.003
Clinical data (no. [%] of subjects)				
Radiographic loosening	1 (50)	31 (30.7)	11 (33.3)	0.78
Revision arthroplasty	2 (100)	99 (98)	7 (21.2)	<0.0001
Timing				
Median (range) time to clinical failure (mo)[b]	44 (30–58)	29 (0–1,211)	14 (0–1,221)	0.08
Median (range) age of prosthesis (mo)[c]	87 (31–143)	47 (6–1,222)	22 (2–1,222)	0.02
No. (%) of subjects with the following case definition:				
Presence of sinus tract[d]	0 (0)	0 (0)	6 (18.2)	<0.0001
Visible purulence at implant site[d]	0 (0)	0 (0)	20 (60.6)	<0.0001
Acute inflammation in periprosthetic tissue (n = 133)[d]	0 (0)	0 (0)	30 (90.9)	<0.0001
No. (%) of subjects with the following laboratory findings:				
Blood leukocyte count of $>10 \times 10^9$/liter (n = 116)	0 (0)	11/90[e] (12.2)	6/26 (23.1)	0.17
ESR of >30 mm/h (n = 106)[f]	0 (0)	6/80 (7.5)	8/26 (30.8)	0.002
CRP concn of >1.0 mg/dl (n = 106)[f]	0 (0)	13/80 (16.3)	12/26 (46.2)	0.002
Synovial fluid leukocyte count of $>1.7 \times 10^9$/liter (n = 28)[g]	ND[h]	1/18 (5.6)	5/10 (50)	0.01
Synovial fluid differential of >65% neutrophils (n = 28)[g]	ND	4/18 (22.2)	7/10 (70)	0.02
No. (%) of subjects with the following underlying joint disorder:				
Osteoarthritis	1 (50)	54/97 (55.7)	13/32 (40.6)	0.14
Trauma	1 (50)	27/97 (27.8)	11/32 (34.4)	0.48
Rheumatoid arthritis	0 (0)	13/97 (13.4)	4/32 (12.5)	1.00
Avascular necrosis	0 (0)	4/97 (4.1)	0 (0)	0.57

[a] By comparison of aseptic failure and definite prosthetic shoulder infection groups.
[b] Time between the last surgery at the implant site and the onset of symptoms.
[c] Time between the last surgery at the implant site and removal of the implant.
[d] Considered a diagnostic criterion for prosthetic shoulder infection.
[e] When the denominator is shown, data were not available for all study subjects.
[f] Cutoffs are from a previous report (21).
[g] Cutoffs are from a previous report (19).
[h] ND, not done.

patient because sonicate fluid was not archived and five patients because they had undergone partial revisions. Of the 136 patients analyzed, 101 had aseptic failure, 33 had definite prosthetic shoulder infection, and 2 had probable prosthetic shoulder infection.

The demographic and clinical characteristics and the laboratory data for the patients are shown in Table 1. There were proportionally more males in the group with definite prosthetic shoulder infection than in the group with aseptic failure (66.6% and 36.6%, respectively; $P = 0.003$). The median age of the prosthesis at the time of revision or resection surgery was lower in the group with definite prosthetic shoulder infection than in the group with aseptic failure (22 and 47 months, respectively; $P = 0.02$). A C-reactive protein (CRP) concentration of >1.0 mg/dl and an erythrocyte sedimentation rate (ESR) of >30 mm/h were more prevalent in the group with definite prosthetic shoulder infection than in the group with aseptic failure (for the CRP concentration, 46.2 and 16.3%, respectively [$P = 0.002$]; for ESR, 30.8 and 7.5%, respectively [$P = 0.002$]).

The sensitivities of sonicate fluid and periprosthetic tissue culture for the detection of definite prosthetic shoulder infection were 66.7% (22/33) and 54.5% (18/33) ($P = 0.046$), respectively, and the specificities were 98.0% (99/101) and 95.1% (96/101) ($P = 0.26$), respectively. Four cases of definite prosthetic shoulder infection were detected by the culture of sonicate fluid but not by the culture of periprosthetic tissue culture; no case of definite prosthetic shoulder infection was detected by the culture of periprosthetic tissue but not by the culture of sonicate fluid. Eight subjects with definite prosthetic shoulder infection had received antimicrobial agents within 4 weeks of revision or resection arthroplasty. One of the eight subjects had negative sonicate fluid and periprosthetic tissue cultures; culture of both types of specimens were positive for the remaining seven subjects.

P. acnes and *Staphylococcus* spp. were the most frequent microorganisms detected by the culture of both periprosthetic tissue and sonicate fluid. *P. acnes* was isolated from 38.9% (7/18) and 40.9% (9/22) of positive cultures of periprosthetic tissue and sonicate fluid, respectively, from subjects with definite prosthetic shoulder infection. A *Propionibacterium* sp. was isolated in cultures of both periprosthetic tissue and sonicate fluid from both patients with probable prosthetic shoulder infection (*P. acnes* and *P. avidum* from one patient each). A *Staphylococcus* sp. was isolated from 50.0% (9/18) and 54.5% (12/22) of cultures of periprosthetic tissue and sonicate fluid,

TABLE 2. Characteristics of patients with definite prosthetic shoulder infection with sonicate fluid cultures positive for *Propionibacterium acnes* versus those of patients with definite prosthetic shoulder infection with sonicate fluid cultures positive for other microorganisms

Characteristic	Sonicate fluid positive for *P. acnes*[a] ($n = 9$)	Sonicate fluid positive for organism(s) other than *P. acnes* ($n = 13$)	*P*
Demographics			
Median (range) age (yr)	56 (44–80)	60 (54–81)	0.22
No. (%) of subjects of female gender	0 (0)	8 (61.5)	0.006
Clinical data (no. [%] of subjects)			
Radiographic loosening	3 (33.3)	3 (23.1)	0.66
Revision arthroplasty	2 (22.2)	1 (7.7)	0.54
Timing			
Median (range) time to clinical failure (mo)[b]	12 (0–213)	12 (0–38)	0.42
Median (range) age of prosthesis (mo)[c]	22 (5–250)	14 (2–51)	0.12
No. (%) of patients with the following case definition:			
Presence of sinus tract[d]	2 (22.2)	3 (23.1)	1.00
Visible purulence at implant site[d]	5 (55.6)	11 (84.6)	0.18
Acute inflammation in periprosthetic tissue[d]	7 (77.8)	13 (100.0)	0.16
No. (%) of patients with the following laboratory findings:			
Blood leukocyte count of $>10 \times 10^9$/liter ($n = 17$)	1/6[e] (16.7)	3/11 (27.3)	1.00
ESR of >30 mm/h ($n = 19$)[f]	1/7 (14.3)	6/12 (50.0)	0.17
CRP concn of >1.0 mg/dl ($n = 19$)[f]	2/7 (28.6)	7/12 (58.3)	0.35
No. (%) of subjects with the following underlying joint disorder:			
Osteoarthritis	2/8 (25)	7 (53.9)	0.37
Trauma	4/8 (50)	2 (15.4)	0.15
Rheumatoid arthritis	0 (0)	3 (23.1)	0.26
Avascular necrosis	0 (0)	0 (0)	

[a] Includes a patient with both *P. acnes* and a *Corynebacterium* sp. growing from sonicate fluid and a patient with both *P. acnes* and a coagulase-negative *Staphylococcus* sp. growing from sonicate fluid.
[b] Time between the last surgery at the implant site and the onset of symptoms.
[c] Time between the last surgery at the implant site and removal of the implant.
[d] Considered a diagnostic criterion for prosthetic shoulder infection.
[e] Where the denominator is shown, data were not available for all study patients.
[f] Cutoffs are from a previous report (21).

respectively, from patients with definite prosthetic shoulder infection.

We compared patients with definite prosthetic shoulder infection with sonicate fluid cultures positive for *P. acnes* with those with definite prosthetic shoulder infection with sonicate fluid cultures that yielded other microorganisms (Table 2). All of the patients with *P. acnes* prosthetic shoulder infection were male, whereas 38.5% of those in the group without *P. acnes* prosthetic shoulder infection were male ($P = 0.006$). The group with *P. acnes* prosthetic shoulder infection showed a trend toward being less likely than the group without *P. acnes* prosthetic shoulder infection to exhibit visible purulence at the implant site (55.6 and 84.6%, respectively; $P = 0.18$) or to have periprosthetic tissue that exhibited acute inflammation in culture (77.8 and 100.0%, respectively; $P = 0.16$), an ESR of >30 mm/h (14.3 and 50.0%, respectively; $P = 0.17$), or a CRP concentration of >1.0 mg/dl (28.6 and 58.3%, respectively; $P = 0.35$).

P. acnes ($n = 7$), coagulase-negative *Staphylococcus* spp. ($n = 5$), *S. aureus* ($n = 4$), *Pseudomonas aeruginosa* ($n = 1$), and *Corynebacterium* sp. ($n = 1$) were isolated from the 18 patients with definite prosthetic shoulder infection and positive periprosthetic tissue cultures. *P. acnes* ($n = 7$), *Staphylococcus epidermidis* ($n = 7$), *S. aureus* ($n = 4$), *P. aeruginosa* ($n = 1$), *P. acnes* plus a *Corynebacterium* sp. ($n = 1$), *P. acnes* plus *S. epidermidis* ($n = 1$), and *Finegoldia magna* ($n = 1$) were isolated from the 22 patients with definite prosthetic shoulder infection and positive sonicate fluid cultures. All but one patient with positive periprosthetic tissue cultures had sonicate fluid cultures with concordant microbiologies. A *Corynebacterium* sp. was detected in that patient by both periprosthetic tissue and sonicate fluid cultures, and *P. acnes* was additionally detected by sonicate fluid culture.

Five patients with aseptic failure had periprosthetic tissue cultures positive for *P. acnes* (as a result of growth from broth only [$n = 2$] or a combination of light growth on a plate and growth from broth [$n = 3$]). Two patients with aseptic failure had positive sonicate fluid cultures; the culture for one patient grew 20 to 50 CFU per plate of a gram-positive bacillus resembling a *Corynebacterium* sp, and the culture for the other patient grew >100 CFU per plate of *P. acnes*. The sonicate fluid from the patient with aseptic failure and >100 CFU per plate of *P. acnes* was positive for *P. acnes* by PCR and immunofluorescence microscopy, and a concomitant single positive periprosthetic tissue specimen was culture positive for *P. acnes*. He had had multiple periprosthetic tissue cultures positive for *P. acnes* at the time of revision arthroplasty with removal of the glenoid component 18 months earlier, and he had been receiv-

ing chronic penicillin suppression until a month prior to surgery, suggesting that this case may have been misclassified. Sonicate fluid from one patient with aseptic failure grew 1 CFU per plate of *P. acnes* on culture, and sonicate fluid from another patient with aseptic failure grew 1 CFU per plate of a coagulase-negative *Staphylococcus* sp. and 1 CFU per plate of a viridans group *Streptococcus* sp. on culture, but these were considered contaminants.

The sensitivities of immunofluorescence microscopy and PCR with sonicate fluid for the detection of definite prosthetic shoulder infection (from any cause) were 39.4% (13/33) and 57.6% (19/33), respectively; the specificities were 98.0% (99/101) and 99.0% (100/101), respectively. The sensitivities of immunofluorescence microscopy and PCR to detect *P. acnes* in sonicate fluids culture positive for this organism were 66.7% (6/9) and 88.9% (8/9), respectively. The sensitivities of immunofluorescence microscopy and PCR to detect *Staphylococcus* spp. in sonicate fluids culture positive for this group of organisms were 58.3% (7/12) and 97.7% (11/12), respectively. When the results of the staphylococcal PCR assay were positive, they completely correlated with those of culture with regard to detection of *S. aureus* versus non-*S. aureus Staphylococcus* spp. The results of all immunofluorescence microscopy and PCR assays positive for *P. acnes* and *Staphylococcus* spp. were concordant with the sonicate fluid culture results. Four definite prosthetic shoulder infection patients with sonicate fluid growth of *S. epidermidis*, one with sonicate fluid growth of *S. aureus*, and three with sonicate fluid growth of *P. acnes* had negative immunofluorescence microscopy results but positive PCR results. One definite prosthetic shoulder infection patient with sonicate fluid growth of *S. epidermidis* had positive immunofluorescence microscopy results but negative PCR results. The patient with a definite prosthetic shoulder infection and sonicate fluid growth of a *Corynebacterium* sp. plus *P. acnes* had a positive immunofluorescence microscopy result but did not have positive PCR results. One subject with sonicate fluid culture-positive definite prosthetic joint infection who had received antimicrobial agents within a month of surgery had negative PCR results, and another had negative immunofluorescence microscopy results.

One patient with definite prosthetic shoulder infection (from whom a *Propionibacterium* sp. was isolated) and 21 patients with aseptic failure (including 14 from whom a *Propionibacterium* sp. and 4 from whom coagulase-negative staphylococci were isolated) had a single positive periprosthetic tissue culture. Had these been considered to represent positive results, the sensitivity and the specificity of periprosthetic tissue culture would have been 19/33 (57.6%) and 70/101 (69.3%), respectively.

DISCUSSION

The results of this study indicate that prosthesis vortexing/sonication in a solid container, which requires a single specimen, is more sensitive than periprosthetic tissue culture (which requires multiple specimens) for the diagnosis of prosthetic shoulder infection, including cases caused by a *Propionibacterium* sp. We do not advocate the sonication of implants in bags (10), as we have previously shown that this is associated with a risk of contamination (20).

P. acnes was associated with two-fifths of the microbiologically confirmed cases of prosthetic shoulder infection. Debeer et al. reported seven prosthetic shoulder infection cases; in only one was *P. acnes* isolated (9). Themistocleous et al. reported four prosthetic shoulder infection cases, but none of those was associated with a *Propionibacterium* sp. (17). Braman et al. reported on seven patients who underwent resection arthroplasty for prosthetic shoulder infection, but none of those infections was associated with a *Propionibacterium* sp. (4). Cuff et al. reported on 17 patients with prosthetic shoulder infection; *P. acnes* alone was isolated from 1 patient and *P. acnes* in conjunction with a *Staphylococcus* sp. was isolated from another patient (8). Among the 29 infected shoulder prostheses described by Coste et al., the most commonly isolated organisms were coagulase-negative staphylococci ($n = 12$) and *P. acnes* ($n = 7$) (7). Those authors either did not report the microbiologic methods used or did not define exactly how such results were used to make a diagnosis of prosthetic shoulder infection. The methods used and the interpretation of the results are important for the accurate classification of *Propionibacterium* prosthetic shoulder infections.

P. acnes can take a long time to grow in culture. Lutz et al. reported that it took an average of 11.4 days for it to grow from specimens associated with arthroplasty and osteosynthesis infections, with the times being shorter in those with early infections than in those with late infections (8.4 and 13.5 days, respectively) (13).

Some authors consider a single periprosthetic tissue culture positive for *P. acnes* to indicate infection. Our data would suggest that this leads to the overdiagnosis of infections. Lutz et al. reported on 12 patients with orthopedic implants (none of which were shoulder implants), each of whom had an average of one specimen positive for *P. acnes* in the context of either prosthesis dysfunction or pseudoarthrosis but who had no signs of sepsis; all the patients were treated exclusively by surgery without antimicrobial treatment and had favorable outcomes (13). Topolski et al. reported on 75 shoulders with (mostly single) positive intraoperative cultures of tissue at revision shoulder arthroplasty without overt infection (18). The most common organism cultured was *P. acnes* ($n = 45$), followed by a coagulase-negative *Staphylococcus* sp. ($n = 17$). Most of the patients were not specifically treated; only 10 of 75 (13.3%) eventually underwent a second revision procedure. Together, our sonicate fluid culture results and the data from Lutz et al. (13) and Topolski et al. (18) indicate that a single periprosthetic tissue specimen positive for *P. acnes* cannot be considered definitive evidence of infection. The corollary to this finding is that single periprosthetic tissue specimens should never be submitted for culture.

Zeller et al. reported on 50 patients with prosthetic hip ($n = 34$), knee ($n = 10$), or shoulder ($n = 6$) infections from whom at least two intraoperative samples taken at revision arthroplasty (capsule, synovial fluid, periprosthetic tissue, bone, etc.) yielded *P. acnes* (24). Approximately one-third had an ESR of >30 mm/h or a CRP concentration of >1.0 mg/dl. Thirty-five patients developed symptoms within 2 years after the index operation, and 15 developed symptoms later than 2 years after the index operation; signs of infection were significantly more frequent in the former group than in the latter group (18/35

and 1/15, respectively; $P < 0.004$) (24). Whether the patients whose onset of symptoms occurred more than 2 years after shoulder implantation actually had an infection or implant colonization (as suggested by Zeller et al. [24]) cannot be definitively determined, as most of the patients were successfully managed with exchange arthroplasty and prolonged administration of antimicrobial agents (24).

All staphylococci isolated from sonicate fluid of patients with definite prosthetic shoulder infection were *S. epidermidis*; this is in contrast to our experience with hip and knee arthroplasties, from which we isolated both *S. epidermidis* and a non-*S. epidermidis Staphylococcus* sp. (22).

Eleven patients in our study who otherwise met the definition of prosthetic shoulder infection had negative sonicate fluid and periprosthetic tissue cultures. Two had been receiving chronic antimicrobial suppression; one had underlying rheumatoid arthritis which may have resulted in histopathologic acute inflammation (the only criterion positive for prosthetic shoulder infection). It is unclear why the cultures were negative for the remainder of the patients.

We applied a higher cutoff for sonicate fluid positivity to the subjects enrolled in the later period of our study than in the earlier period of our study as a result of the introduction of sonicate fluid concentration prior to culture. We previously determined that the ideal cutoff value for unconcentrated sonicate fluid positivity for hip and knee implants was ≥5 CFU of the same organism detected on the aerobic and/or anaerobic sheep blood agar plate (22). The addition of the concentration step resulted in a 20-fold concentration of sonicate fluid, so an argument that the cutoff for positivity should be reset to 100 CFU per plate could be made. However, the use of a cutoff of 20 CFU per plate with concentrated sonicate fluid resulted in a sensitivity and a specificity similar to those achieved with a cutoff of 5 CFU per plate with unconcentrated sonicate fluid when it was applied to hip and knee implants (14). Furthermore, in a study of spinal implants from 122 study subjects, all except one of the sonicate fluid cultures with positive results had ≥100 CFU per plate; the sonicate fluid culture that was the exception yielded 20 to 50 CFU per plate, and the sonicate fluid was from a patient who was receiving chronic antimicrobial suppression for a prior implant infection and who had positive periprosthetic tissue cultures, suggesting that this should be considered a positive result (M. Fernandez Sampedro, M., P. M. Huddleston, K. E. Piper, M. J. Jacobson, M. B. Dekutoski, M. J. Yaszemski, B. L. Currier, J. N. Mandrekar, D. R. Osmon, A. McDowell, S. Patrick, J. M. Steckelberg, and R. Patel, submitted for publication). In the current study, there were seven patients with prosthetic shoulder infections with sonicate fluid cultures that yielded 20 to 99 CFU per plate. One probable infection case had 20 CFU of *P. acnes* per plate, with concomitant positive periprosthetic tissue culture results. Two subjects had 20 to 50 CFU of *P. acnes* per plate, three had 51 to 100 CFU of *S. aureus* per plate, and one had 20 to 50 CFU of a coagulase-negative *Staphylococcus* sp. per plate and 20 to 50 CFU of *P. acnes* per plate. The remainder of the patients had sonicate fluid cultures with the growth of ≥100 CFU per plate. Together, the results of studies of hip, knee, shoulder, and spine implants indicate that when concentrated sonicate fluid is cultured, a cutoff of ≥20 CFU per plate should be applied to determine a clinically significant result.

In our study, patients with sonicate fluid cultures positive for *P. acnes* were more likely to be male than patients with sonicate fluid cultures positive for other microorganisms. Others have reported that male gender is a risk factor for *P. acnes* arthritis (3) or *P. acnes* shoulder infection (12).

Most authors consider *Propionibacterium* infections to be of endogenous origin. Interestingly, Berthelot et al. determined that the performance of surgery earlier in the day and an increased duration of surgery were risk factors for outbreak-associated shoulder arthritis due to *P. acnes* (3). Modification of ventilation and implementation of improved cleaning in the operating room were associated with an end of the outbreak (3). Pinpointing the source of a *Propionibacterium* sp. causing prosthetic shoulder infections is important in preventing such cases from occurring.

It has been suggested that the *Propionibacterium* PCR is insensitive, possibly due to poor DNA extraction as a result of the organism's cell wall (12); this was not our experience. The assay that we evaluated was specific and missed only a single case in which the sonicate fluid culture was positive for *P. acnes*. Given the time that it takes to isolate *Propionibacterium* spp. in culture, the clinical application of PCR may be helpful. *Staphylococcus* spp. (*S. epidermidis*, $n = 8$; *S. aureus*, $n = 4$) were detected in 54.5% (12/22) of definite prosthetic shoulder infection sonicate fluid cultures. A *Staphylococcus* PCR performed with sonicate fluid was specific and missed a single case in which the sonicate fluid culture was positive for *S. epidermidis*. Together, *P. acnes* and *Staphylococcus* spp. accounted for 90.9% of the microbiology of positive sonicate fluid cultures among subjects with definite prosthetic shoulder infections; 90.5% of these cases were detected by PCR. The clinical application of these rapid assays may be helpful with the detection of the most common microorganisms causing prosthetic shoulder infection.

Finally, it should be noted that ESR and the CRP concentration are insensitive indicators for *Propionibacterium* prosthetic shoulder infection. This finding, combined with the infrequent presence of a sinus tract communicating with the prosthesis or visible purulence at the implant site, reinforce the need for accurate diagnostics for *Propionibacterium* prosthetic shoulder infections.

In summary, we have shown that sonicate fluid culture is useful for the diagnosis of prosthetic shoulder infection and that *Propionibacterium* spp., which cause a substantial proportion of prosthetic shoulder infections, are accurately detected in sonicate fluid cultures. Single periprosthetic tissue cultures positive for a *Propionibacterium* sp. are nonspecific for the diagnosis of prosthetic shoulder infection.

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R. Patel reports having an unlicensed U.S. patent pending for a method and an apparatus for sonication but has forgone her right to receive royalties in the event that the patent is licensed. No other potential conflicts of interest were disclosed.

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