JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2006, p. 628–631 Vol. 44, No. 2
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.2.628–631.2006

Sonication of Explanted Prosthetic Components in Bags for Diagnosis of Prosthetic Joint Infection Is Associated with Risk of Contamination

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Received 14 November 2005/Accepted 16 November 2005

Explanted orthopedic implants from 54 patients with aseptic failure and 24 patients with prosthetic knee or hip infection were sonicated in polyethylene bags. The sensitivities of periprosthetic tissue and sonicate fluid cultures for the diagnosis of prosthetic joint infection were 54% and 75%, whereas the specificities were 98% and 87%, respectively. Sonication in bags improved bacterial recovery from the surface of orthopedic implants; however, it lacked specificity, due to bag leakage.

Prosthetic joint implantation has improved the quality of life for many individuals by restoring satisfactory, pain-free joint function (8). The most common complication of joint replacement is aseptic failure, followed by prosthetic joint infection (PJI) (5, 6). Since treatment strategies are fundamentally different, it is important to accurately distinguish these two clinical entities (11). Periprosthetic tissue is the standard specimen cultured intraoperatively for the diagnosis of PJI but does not sample the prosthesis surface, to which microorganisms are attached and grow in biofilms (2).

Tunney et al. used sonication of explanted hip prostheses to dislodge adherent bacteria (9, 10). Their study was limited by the lack of a well-formulated definition of PJI, incomplete clinical and histopathologic data, and missing information on antimicrobial treatment prior to revision arthroplasty. Nevertheless, they suggested that the incidence of PJI is underestimated by current culture detection methods. We therefore performed a study to determine whether their sonication method, aimed at detecting bacteria in biofilms on the prosthesis surface, can improve the diagnosis of PJI.

Between July 1998 and August 2003, patients at the Mayo Clinic, Rochester, MN, undergoing total knee or hip prosthesis removal for aseptic failure or presumed infection were prospectively enrolled. Subjects were excluded if prosthetic components were incompletely removed, obvious contamination of an explanted component occurred in the operating room, fewer than two periprosthetic tissue specimens were collected for culture, or fungal infection occurred. The study was approved by the Mayo Clinic Institutional Review Board; all patients provided informed consent.

Prosthetic joint infection was diagnosed if at least one of the following was present (1): (i) visible purulence of synovial fluid or area surrounding the prosthesis (as determined by the surgeon), (ii) acute inflammation on histopathologic examination of permanent periprosthetic tissue sections (as determined by the clinical pathologist), or (iii) a sinus tract communicating with the prosthesis. Aseptic failure was defined as prosthesis failure not meeting criteria for PJI. Organisms were defined as causative if the same organism was cultured from a patient meeting the definition of PJI from at least two periprosthetic tissue specimens (with the exception of *Staphylococcus aureus*, which was considered causative even when isolated from a single specimen) or from synovial fluid. Prior antimicrobial therapy was defined as receipt of antimicrobials for at least 2 weeks, completed within 3 days before surgery. The status of the newly implanted prostheses was assessed 2 years after implantation.

Preoperatively, synovial fluid was aspirated at the discretion of the surgeon. Intraoperatively, tissue specimens with the most obvious inflammatory changes were collected for microbiological and histopathologic studies. Removed prosthetic components were placed in sterile 12- by 12-inch polyethylene bags (Bitran PE 3 Mil; COM-PAC International, Carbondale, IL). The tibial component and tibial tray (from knee prostheses) or acetabular component and liner (from hip prostheses) were placed in one bag and the femoral component (from knee and hip prostheses) and patellar button (from knee prostheses) in another. The bags were placed into an anaerobic jar and processed within 4 h of prosthesis removal. Prior to usage, polyethylene bags were sterilized with 1 kGy in a self-contained ^{137}Cs gamma-irradiator (Mark I; J. L. Shepherd, San Fernando, CA).

In the microbiology laboratory, 100 ml of Ringer's solution (25% [vol/vol]) containing L-cysteine (0.05% [wt/vol]) was added to each bag. Sonication of double-bagged prosthetic components was performed with a continuous sinusoidal wave ultrasound cleaner (Aquasonic 750T; VWR Scientific, West Chester, PA) for 5 min at room temperature. Ultrasound parameters were measured using a calibrated hydrophone (type 8103; Brüel and Kjær, Naerum, Denmark). No differences in frequency (40 ± 5 kHz) or power density (0.22 ± 0.05 W/cm^2) were observed over the study period at various locations within the ultrasound bath, including inside and outside the bags. During sonication, the temperature of the tank water increased <0.5°C.

Synovial fluid was inoculated in aliquots of 0.1 ml to aerobic

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TABLE 1. Characteristics of study patients and results of microbiological studies

Characteristic[a]	Result for patients with:		*P*
	Aseptic failure ($n = 54$)	PJI ($n = 24$)	
Median age, yr (range)	71.5 (40–88)	71 (50–89)	0.953
Male gender	29 (54)	13 (54)	0.970
Revision arthroplasty	7 (13)	8 (33)	0.059
Median age of prosthesis, mo (range)[b]	71 (1.4–307.3)	18 (0.3–170.7)	0.0006
Presence of sinus tract[c]	0	6 (25)	0.0005
Visible purulence of synovial fluid ($n = 63$)[c]	0/46	10/17 (59)	<0.0001
Visible purulence at implant site[c]	0	21 (88)	<0.0001
Acute inflammation in periprosthetic tissue ($n = 74$)[c]	0/52	16/22 (73)	<0.0001
Laboratory findings			
Synovial fluid leukocytes, $>1.7 \times 10^9$/liter[d]	5/30 (17)	15/15 (100)	<0.0001
Synovial fluid differential, >65% neutrophils[d]	1/30 (3)	15/15 (100)	<0.0001
Cultures			
Synovial fluid ($n = 64$)	0/47	13/17 (77)	<0.0001
Periprosthetic tissue (≥1 specimen positive)	5 (9)	18 (75)	<0.0001
Periprosthetic tissue (≥2 specimens positive)	1 (2)	13 (54)	<0.0001
Sonicate fluid	7 (13)	18 (75)	<0.0001

[a] Data are numbers (percentages) of patients, unless otherwise indicated. Where the number of subjects is shown, data were not available for all study subjects.
[b] Time between last surgery at the implant site and removal of the implant.
[c] Considered a diagnostic criterion for PJI.
[d] Cutoff taken from reference 7.

blood, chocolate, and anaerobic blood agar and into thioglycolate broth (BD Diagnostic Systems, Sparks, MD). Residual volumes of synovial fluid >0.5 ml were inoculated into a BACTEC Peds Plus/F bottle and incubated in a BACTEC 9240 instrument (BD Diagnostic Systems) for 5 days, as previously described (3). Tissue specimens were individually homogenized in 3 ml brain heart infusion broth for 1 min (using a mortar and pestle, if bone was present), and the homogenate was inoculated in aliquots of 0.5 ml to aerobic blood, chocolate, and anaerobic blood agar and into thioglycolate broth. For purposes of comparing quantitative yields of sonicate fluid versus those of tissue cultures, one periprosthetic tissue specimen was subjected to quantitative culture. Aliquots of 0.5 ml sonicate fluid were plated onto each of five aerobic and five anaerobic blood agar plates. A positive sonicate fluid culture was defined as growth of any organism on at least four of five plates from any bag (9). All aerobic cultures were incubated at 35 to 37°C in 5 to 7% CO_2 for 5 days and anaerobic cultures at 35 to 37°C in anaerobic conditions for 7 days and examined daily. Each unique colony was classified using routine microbiological techniques. Comparisons between variables were performed by the Wilcoxon rank sum, χ^2, or Fisher exact tests, as appropriate, using the statistical software package JMP (version 6.0; SAS Institute, Inc., Cary, NC).

After exclusion of patients with incomplete implant removal ($n = 3$), fewer than two periprosthetic tissue specimens submitted ($n = 2$), and *Sporothrix schenckii* infection ($n = 1$), 78 patients with total knee ($n = 68$) or hip prostheses ($n = 10$) were studied; 54 had aseptic failure and 24 PJI (Table 1). The groups were similar in terms of age, gender, type of prosthesis, and frequency of radiographic loosening. The predominant reasons for primary arthroplasty had been osteoarthritis ($n = 64$), bone fracture ($n = 9$), inflammatory joint disease ($n = 3$), and avascular necrosis ($n = 2$). The sensitivities of periprosthetic tissue (considering at least two specimens positive) and sonicate fluid cultures were 54% (95% confidence interval [CI], 33 to 75%) and 75% (95% CI, 53 to 95%), respectively, whereas the specificities of the same specimens were 98% (95% CI, 90 to 100%) and 87% (95% CI, 75 to 95%), respectively. For patients with PJI, all six negative sonicate fluid cultures were obtained from those patients receiving antimicrobial agents, whereas only 7 of 18 patients (39%) with positive sonicate fluid cultures had taken antimicrobial agents ($P < 0.02$). Excluding patients who had received antimicrobial agents, the sensitivities of sonicate fluid, periprosthetic tissue, and synovial fluid culture were 100% (95% CI, 81 to 100%), 73% (95% CI, 48 to 94%), and 90% (95% CI, 66 to 100%), respectively.

Causative microorganisms were isolated from synovial fluid and/or periprosthetic tissue cultures from 18 patients (75%) with PJI. Among patients with aseptic failure, bacteria were isolated in cultures from five patients (coagulase-negative *Staphylococcus* species [$n = 3$], *Corynebacterium* species [$n = 1$], and *Propionibacterium* species [$n = 1$]). No synovial fluid culture was positive for patients with aseptic failure. The microbiology of sonicate fluid cultures was concordant with that of synovial fluid and periprosthetic tissue cultures where these were positive.

In patients with aseptic failure, seven sonicate fluid cultures were positive (*Propionibacterium* species [$n = 2$] and nonfermenting gram-negative bacilli [$n = 5$]). None of these results correlated with results of tissue cultures. The nonfermenting gram-negative bacilli appeared to relate to contamination with waterborne microorganisms as a result of bag leakage, as suggested by growth of the same organisms from water in the ultrasound bath. Visible bag leakage was apparent in several cases, presumably due to penetration of the bags by sharp bone and cement fragments and/or the effects of irradiation and/or

TABLE 2. Culture results of study subjects with PJI

Patient group	Sample no.	Result for cultures from[a]: Synovial fluid	Periprosthetic tissue			Sonicate fluid	
		Organism	Organism	No. positive/ no. taken	CFU/g tissue[b]	Organism	CFU/ml sonicate[c]
Subjects who received antimicrobial therapy before surgery	1	ND	*Corynebacterium* sp.	2/4	4	*Corynebacterium* sp.	250
	2	CNS	CNS	1/3	>200	CNS	>2,000
	3	CNS	CNS	6/6	11	CNS	>2,000
	4	ND	CNS	3/3	2	CNS	>2,000
	5	ND	*Corynebacterium* sp.	1/3	5	Neg	0
	6	Neg	*S. aureus*	1/3	38	Neg	0
	7	Neg	Neg	0/5	0	Neg	0
	8	*S. aureus*	*S. aureus*	3/3	>200	*S. aureus*	>2,000
	9	Neg	CNS	4/4	9	CNS	150
	10	*S. aureus*	*S. aureus*	1/3	36	*S. aureus*	>2,000
	11	ND	Neg	0/4	0	Neg	0
	12	ND	Neg	0/3	0	NF-GNB	850
	13	ND	Neg	0/3	0	Neg	0
Subjects who did not receive antimicrobial therapy before surgery	14	CNS	CNS	3/3	20	CNS	>2,000
	15	VGS	Neg	0/3	0	VGS	>2,000
	16	CNS	CNS	3/4	2	CNS	750
	17	*S. aureus*	*S. aureus*	3/3	>200	*S. aureus*	>2,000
	18	CNS	Neg	0/2	0	CNS	>2,000
	19	CNS	CNS	3/3	ND	CNS	>2,000
	20	CNS	CNS	3/3	17	CNS	>2,000
	21	CNS	CNS	3/3	>200	CNS	>2,000
	22	*S. aureus*	*S. aureus*	3/3	>200	*S. aureus*	110
	23	Neg	CNS	1/3	2	CNS	16
	24	ND	CNS	3/4	50	CNS	>2,000

[a] Abbreviations: Neg, negative; CNS, coagulase-negative *Staphylococcus* sp.; VGS, viridans group *Streptococcus* sp.; NF-GNB, nonfermenting gram-negative bacillus; ND, not done.
[b] Mean CFU per gram of periprosthetic tissue.
[c] Mean CFU per ml of sonicate fluid.

sonication on the bags. In patients with PJI, the number of CFU of bacteria from positive sonicate fluid cultures (per ml sonicate fluid) was greater than the number from the respective periprosthetic tissue culture (per gram tissue) ($P = 0.002$) (Table 2). At follow-up 2 years after arthroplasty, none of the five aseptic-failure patients with positive periprosthetic tissue cultures and none of the seven aseptic-failure patients with positive sonicate fluid cultures had developed PJI or aseptic failure.

Results of this study suggest that sonication of the removed implants improves microbial recovery in comparison to conventional periprosthetic tissue culture. Sonicate fluid cultures detected infecting organisms in higher numbers than did periprosthetic tissue cultures (Table 2). Importantly, all six false-negative sonicate fluid cultures occurred with patients taking antimicrobial agents, emphasizing the importance of discontinuation of antimicrobial therapy prior to specimen collection. For 5 of 18 patients with PJI, sonicate fluid cultures grew at least 10-fold-greater numbers of bacteria from one bag than from the other (data not shown), suggesting either that PJI can be a focal infection involving only some component or that trapping of air between the double bags hindered transmission of ultrasound to the implant surface.

Unfortunately, bag sonication was suboptimal because of false-positive cultures, apparently related to bag leakage. The finding of *Propionibacterium* species in sonicate fluid cultures from two aseptic-failure cases (one hip and one knee prosthesis) is concordant with the findings of Tunney et al. (9), although in their study this organism was found more frequently. As has been suggested by others (4), the role of *Propionibacterium* species in the pathogenesis of aseptic implant failure remains to be determined.

In conclusion, methods aimed at detecting biofilm bacteria on prosthesis surfaces improve bacterial recovery in comparison to conventional tissue cultures. However, sonication of prosthetic components in bags lacks specificity due to bag leakage and subsequent risk of microbial contamination. We are currently performing a study processing removed orthopedic implants in solid containers.

We thank John G. Hughes, Don Baltes, and Dean S. Strain for their technical assistance, Jayawant Mandrekar for statistical support, and K. Krishnan Unni for reviewing the histopathology of the periprosthetic tissue sections.

This study received financial support from the Mayo Foundation, Rochester, MN; the Orthopaedic Research and Education Foundation; the Swiss National Science Foundation (grant number BS81-64248); and the Roche Research Foundation.

REFERENCES

1. **Berbari, E. F., A. D. Hanssen, M. C. Duffy, J. M. Steckelberg, D. M. Ilstrup, W. S. Harmsen, and D. R. Osmon.** 1998. Risk factors of prosthetic joint infection: case-control study. Clin. Infect. Dis. **27:**1247–1254.
2. **Donlan, R. M.** 2001. Biofilm formation: a clinically relevant microbiological process. Clin. Infect. Dis. **33:**1387–1392.
3. **Hughes, J. G., E. A. Vetter, R. Patel, C. D. Schleck, S. Harmsen, L. T. Turgeant, and F. R. Cockerill III.** 2001. Culture with BACTEC Peds Plus/F bottle compared with conventional methods for detection of bacteria in synovial fluid. J. Clin. Microbiol. **39:**4468–4471.
4. **Nguyen, L. L., C. L. Nelson, M. Saccente, M. S. Smeltzer, D. L. Wassell, and S. G. McLaren.** 2002. Detecting bacterial colonization of implanted orthopaedic devices by ultrasonication. Clin. Orthop. Relat. Res. **403:**29–37.
5. **Rand, J. A., and D. M. Ilstrup.** 1991. Survivorship analysis of total knee arthroplasty. Cumulative rates of survival of 9200 total knee arthroplasties. J. Bone Jt. Surg. Am. Vol. **73:**397–409.
6. **Segawa, H., D. T. Tsukayama, R. F. Kyle, D. A. Becker, and R. B. Gustilo.** 1999. Infection after total knee arthroplasty. A retrospective study of the treatment of eighty-one infections. J. Bone Jt. Surg. Am. Vol. **81:**1434–1445.
7. **Trampuz, A., A. D. Hanssen, D. R. Osmon, J. Mandrekar, J. M. Steckelberg, and R. Patel.** 2004. Synovial fluid leukocyte count and differential for diagnosis of prosthetic knee infection. Am. J. Med. **117:**556–562.
8. **Trampuz, A., J. M. Steckelberg, D. R. Osmon, F. R. Cockerill, A. D. Hanssen, and R. Patel.** 2003. Advances in the laboratory diagnosis of prosthetic joint infection. Rev. Med. Microbiol. **14:**1–14.
9. **Tunney, M. M., S. Patrick, M. D. Curran, G. Ramage, D. Hanna, J. R. Nixon, S. P. Gorman, R. I. Davis, and N. Anderson.** 1999. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J. Clin. Microbiol. **37:**3281–3290.
10. **Tunney, M. M., S. Patrick, S. P. Gorman, J. R. Nixon, N. Anderson, R. I. Davis, D. Hanna, and G. Ramage.** 1998. Improved detection of infection in hip replacements. A currently underestimated problem. J. Bone Jt. Surg. Br. Vol. **80:**568–572.
11. **Zimmerli, W., A. Trampuz, and P. E. Ochsner.** 2004. Prosthetic-joint infections. N. Engl. J. Med. **351:**1645–1654.