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Letters to the Editor

Evaluation of Conventional Microbiological Procedures and Molecular Genetic Techniques for Diagnosis of Infections in Patients with Implanted Orthopedic Devices[▽]

The incidence of infections resulting in prosthetic joint failure is estimated to vary between 0.5% and 2% (2, 6). It has been suggested that routine specimen collection techniques may not be adequate for a prosthesis surface to which biofilm-embedded bacteria are attached. Ultrasonication of prostheses followed by analysis of the dislodged material (sonicate) was suggested to improve the detection of prosthetic hip infections (5). Tunney et al. showed that culture of periprosthetic tissue samples was positive in 4% of cases, increasing in sonicate cultures (22% positive). In comparison, nonculture methods were positive in 63% of cases when sonicates and immunofluorescence were used and in 72% of cases with sonicates and PCR (5). Trampuz et al. reported that culture of sonicates of explanted prosthetic components improved bacterial cultural recovery, although this was associated with the risk of contamination (3). The aim of the present study was to evaluate the benefit of ultrasonication for the recovery of microorganisms from implants by using both cultural and gene amplification techniques.

From August 2003 to August 2004, 69 orthopedic implants (38 hip prostheses, 21 shoulder prostheses, 9 knee prostheses, and 1 ankle prosthesis) were removed at revision operations for aseptic failure or presumed infection. According to an in-house standardized clinical score and on the basis of clinical and laboratory information, a prosthetic joint infection was rated as definitive, probable, possible, or rejected as diagnosis. Intraoperative tissue specimens were sampled (an average of four tissue samples per implant removed) in theater and cultured using standard techniques. Removed implants were placed aseptically in sterile polyethylene bags to which Ringer solution (100 ml) was added, and the bags were heat sealed. The entire bag was then placed in a sonication bath (USC 900 TH; VWR International, Dietikon, Switzerland) and sonicated at 45 kHz at 200 W for 10 min.

TABLE 1. Clinical infection scores versus results for microbiological investigations

Condition and patient no. or quantity	Clinical infection	Microbiological result[a] for:		
		Conventional culture	Sonication	
			Culture	PCR
Clinical infection with positive conventional culture and positive or negative postsonication culture (n = 11)				
1	Definitive	*S. aureus*	*S. aureus*	*S. aureus*
2	Definitive	*S. aureus*[c]	*S. aureus*	*S. aureus*
3	Definitive	*E. cloacae*[d]	*E. cloacae*	*E. cloacae*
4	Definitive	*S. aureus*	*S. aureus*	*S. aureus*
5	Probable	CNS	CNS	*S. epidermidis*
6	Probable	*C. albicans*	*C. albicans*	Neg.[b]
7	Probable	CNS	CNS	CNS
8	Probable	*Peptostreptococcus* sp.[d]	*Peptostreptococcus* sp.[d]	*S. epidermidis*
9	Definitive	CNS	Neg.[d]	*S. epidermidis*
10	Probable	CNS	Neg.[d]	CNS
11	Probable	*P. acnes*	Neg.	*P. acnes*
Clinical infection with negative conventional culture and positive postsonication culture (n = 2)				
12	Definitive	Neg.	*P. acnes*[d]	*P. acnes*
13	Definitive	Neg.	*Bacillus clausii*	CNS
Clinical infection with negative conventional culture and negative postsonication culture (n = 1)				
14	Definitive	Neg.	Neg.[d]	Neg.
Clinical infection absent with positive culture (n = 2)				
15	Possible	*P. acnes*	*P. acnes*[d]	Neg.
16	Possible	Neg.[d]	CNS	Neg.
Clinical infection absent with negative culture (n = 53)				
n = 25	Possible	Neg.	Neg.[e]	Neg.
n = 28	Rejected	Neg.[g]	Neg.[f]	Neg.

[a] In cases where enrichment broth resulted in isolation of additional microorganisms, the respective species is given in the footnote. CNS, coagulase-negative staphylococci; Neg., negative.
[b] As broad-range eubacterial primers were used in PCR, these primers do not recognize *Candida albicans* ribosomal DNA.
[c] *Bacillus* sp. cultivated from enrichment broth.
[d] Coagulase-negative staphylococci cultivated from enrichment broth.
[e] Coagulase-negative staphylococci cultivated from enrichment broth in 6 patients.
[f] Coagulase-negative staphylococci cultivated from enrichment broth in 5 patients.
[g] Coagulase-negative staphylococci (n = 3), *P. acnes* (n = 2), and *Peptostreptococcus* sp. (n = 1) cultivated from enrichment broth.

Sonicates were inoculated on aerobic and anaerobic agar plates (0.5 ml) and in enrichment cultures (5.0 ml). In addition, sonicates were subjected to broad-range eubacterial PCR by centrifuging 50 ml of the sonicate at 4,000 × *g* for 30 min; after removal of the supernatant, the pellet was used for DNA extraction, purification, amplification, gel electrophoresis, and sequence analysis as described previously (1). For tissue samples, bacterial growth in at least one sample following direct plating or enrichment growth in more than one sample was considered positive, and enrichment growth in a single tissue was considered negative; direct bacterial growth of the sonicate on at least one of the inoculated plates was considered positive, and enrichment growth only was considered negative. Typical water contaminants, e.g., *Acidovorax* spp., *Dechloromonas* spp., and *Ralstonia* spp., cultured or detected by PCR from sonicates were not taken into account.

According to the clinical score, a prosthetic joint infection was present in 14 of the 69 patients. Eleven of 14 had positive cultures for tissue samples; 13/14 were found to have positive cultures when the results from sonicate cultures were considered in addition. Sonicate PCR was positive in 12/13 and negative in 1/13 (excluding 1 infection due to *Candida albicans*) (Table 1). Ultrasonication improved the sensitivity of cultures from 78.6% to 92.9%. Processing ultrasonicated implants by PCR resulted in a sensitivity of 92.3% compared to 71.4% by culture. The sensitivity of PCR of sonicates was similar to that of the combined cultures of periprosthetic tissues and sonicates (92.3% versus 92.9%). According to the clinical score, infection was considered possible, but unlikely, in 27 patients and rejected as diagnosis in 28 patients; altogether, in 55 patients infection was not suspected from a clinical point of view. For these patients, conventional cultures of intraoperative tissue samples and sonicate cultures were false positive in 1/55 and 2/55 cases, respectively (Table 1). The specificity of PCR of sonicates was found to be 100% when typical waterborne bacteria found by PCR in four sonicates and four sonicates with mixed sequences were not taken into consideration.

The most important procedure in laboratory diagnosis of prosthesis-related infection is a technique for sampling several periprosthetic tissues in theater; ultrasonication of prostheses adds a moderate, albeit not significant, benefit in sensitivity (92.9% versus 78.6%). In the study by Trampuz et al. (3), ultrasonication was found to increase sensitivity at the expense of specificity. A rigorous interpretation of microbiological findings combined with PCR allowed us to avoid this problem at large. Given the significant risk of contamination during the ultrasonication procedure, e.g., due to bag leakage, we deliberately decided to regard typically waterborne microorganisms as contaminants. Despite all these measures, two false-positive ultrasonication cultures were found in the 55 patients where infection was not suspected from a clinical point of view. In our hands, PCR of sonicates was found to be an exquisitely specific parameter, which may aid in improving the positive predictive value of cultural microbiological investigations. In the case of a clinically possible, but unlikely, or rejected infection, postsonication PCR may be helpful in recognizing false-positive cultural results of periprosthetic tissues.

Based on our preliminary results, we currently use the following diagnostic procedure: (i) according to the clinical score, patients are categorized into definitive, probable, possible, and rejected infection groups; (ii) tissue samples and implants are obtained for laboratory investigations; (iii) in the case of a definitive or probable infection, tissue samples and the sonicated implant are subjected to cultural investigations; and (iv) in the case of a possible or rejected infection, tissue samples are subjected to cultural investigations, and the sonicate is used for PCR in the case of a positive culture to increase specificity. While the manuscript was in the review process, Trampuz et al. reported that culture of samples obtained by sonication of prostheses was significantly more sensitive than conventional periprosthetic tissue culture (4). What could explain this difference? Trampuz et al. did not distinguish between bacterial growth upon direct plating and enrichment growth. Specificity was thus achieved at the expense of sensitivity by considering positive only cases with at least two positive tissue culture results. We realize that the main limitation of our study is the lack of histopathology, as this is not part of the diagnostic workup in place. Despite this, our study indicates that PCR adds little, if any, gain in sensitivity. Larger studies in combination with a clinical score are required to establish the optimal and most cost-efficient diagnostic procedure.

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