Antimicrobial-Loaded Bone Cement Does Not Negatively Influence Sonicate Fluid Culture Positivity for Diagnosis of Prosthetic Joint Infection

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We compared culture results to investigate the influence of antimicrobial-loaded cement on sonicate fluid culture positivity for the diagnosis of prosthetic joint infection. Fifty-four subjects were assessed. The sensitivities of sonicate fluid culture were 77.8% (14 of 18) in subjects with an antimicrobial-loaded cemented prosthesis and 58.3% (21 of 36) in subjects with an antimicrobial-free prosthesis.

With the increasing number of lower-extremity total joint arthroplasty surgeries, there has been an increase in the number of prosthetic joint infections (PJIs) (1). Organisms associated with PJI attach to the implant, polymethylmethacrylate (PMMA), and/or bone surfaces, where they grow in biofilms. Antimicrobial-loaded PMMA is used in the prevention and treatment of PJI (2, 3). We previously reported that culture of fluid obtained by sonication of removed implants is more sensitive than culture of conventional periprosthetic tissue for the diagnosis of PJI (4). Previous antimicrobial exposure has been reported to have a negative effect on microbiologic diagnosis (4, 5). Moreover, it has been shown that *in vitro* application of pulsed ultrasound leads to increased release of gentamicin from antimicrobial-loaded bone cement (6). Therefore, the aim of this study was to determine, by using a retrospective review of clinical cases, whether antimicrobial-loaded PMMA influences sonicate fluid culture positivity.

PJI was diagnosed using Infectious Diseases Society of America guidelines if at least one of the following was present: a sinus tract communicating with the prosthesis, purulence noted at the time of surgery, or acute inflammation detected on intraoperative frozen section histology (7). Patients with removal of spacers during the two-stage exchange process or subjects who were <18 years old as well as those with the criteria noted below were excluded. Between April 2006 and August 2015, 362 patients with confirmed PJI undergoing removal of a total knee or hip prosthesis at Mayo Clinic, Rochester, Minnesota, were enrolled; 227 were excluded because they had received antimicrobial therapy within 4 weeks before the collection of the implant for culture, 80 were excluded because of insufficient medical record information, and one who had a *Mycobacterium chelonae* infection was excluded. A total of 54 patients were enrolled, and all had undergone removal of a single prosthesis.

Tissue culture was performed on 2 to 7 periprosthetic tissue specimens per patient. Implants were cultured according to a previously described protocol, which included sonication, vortexing, and concentration (8). Briefly, the container was vortexed for 30 s and subjected to sonication (40 kHz and 320 mW/cm² for 5 min). Sonicate fluid culture positivity was determined according to a previously described method (8). The Mann-Whitney U test was used to compare the continuous variables, and the χ^2 test was used

to compare the categorical variables. All *P* values were two tailed, and a *P* value of <0.05 was considered statistically significant.

The sensitivity of sonicate fluid culture of removed prostheses (35/54, 64.8%) was superior to that of periprosthetic tissue culture (31/54, 57.4%; *P* < 0.001). Eighteen patients had antimicrobial-loaded PMMA (antimicrobial-PMMA) present at the time of prosthesis removal, and 36 patients (antimicrobial-free) had either an uncemented prosthesis (*n* = 20) or cement without antimicrobials (*n* = 16). Among the 21 patients with a knee prosthesis, 13 had antimicrobial-PMMA, and among the 33 subjects with a hip prosthesis, 5 had antimicrobial-PMMA (*P* < 0.001) (Table 1). The median time between implantation and explantation was 34 months in the antimicrobial-PMMA group and 23 months in the antimicrobial-free group. Among the 35 sonicate fluid culture-positive cases, 7 presented within 12 months after implantation, 9 between 13 and 24 months after implantation, and 19 beyond 24 months of implantation. The sensitivities of sonicate fluid culture were 77.8% in the antimicrobial-PMMA group and 58.3% in the antimicrobial-free group (Table 1). Visible purulence and higher blood leukocyte count, erythrocyte sedimentation rate, and C-reactive protein level were associated with sonicate fluid culture positivity (Table 2). The most common microorganisms isolated were coagulase-negative *Staphylococcus* species and *Staphylococcus aureus*. Antimicrobials used in the PMMA and isolated organisms are shown in Table 3. It should be noted that Gram-positive cocci were isolated from vancomycin-loaded PMMA.

In vivo or *in vitro* studies of antimicrobial release kinetics from PMMA have reported a bimodal release curve with high initial concentrations followed by sustained release (9, 10). We applied

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TABLE 1 Characteristics and comparison of microbiologic data of 54 patients with prosthetic joint infection in this study

Variable	Antimicrobial-loaded prosthesis (n = 18) (%)	Antimicrobial-free prosthesis (n = 36) (%)	P value
Patient demographics			
Age (mean $\pm$ SD) (yr)	67.9 $\pm$ 13.5	65.6 $\pm$ 14.3	0.56
Males (no. [%])	13 (72)	21 (58)	0.85
Reason for primary arthroplasty (no. [%])			
Osteoarthritis	6 (33)	24 (67)	
Bone fracture or trauma	3 (17)	4 (11)	
Rheumatoid arthritis	2 (11)	1 (3)	
Congenital abnormality	1 (6)	1 (3)	
Avascular bone necrosis	0	1 (3)	
Bone neoplasia	2 (11)	0	
Unknown	4 (22)	5 (14)	
Site of arthroplasty (no. [%])			<0.001
Knee	13 (72)	8 (22)	
Hip	5 (28)	28 (78)	
Risk factors for prosthetic joint infection (no. [%])			
Diabetes mellitus	1 (6)	6 (17)	
Immunosuppressive agents (steroids, methotrexate)	2 (11)	3 (8)	
Visible purulence (no./total no.)	16/18	28/36	
Acute inflammation in tissue (no./total no.)	11/13	22/28	
Presence of sinus tract (no./total no.)	2/18	2/36	
Preoperative laboratory data			
Hemoglobin (mean $\pm$ SD) (mg/dl)	11.4 $\pm$ 1.5	12.2 $\pm$ 1.9	0.11
Blood leukocyte count (mean $\pm$ SD) (per mm ³)	8,210 $\pm$ 2,720	9,430 $\pm$ 4,972	0.33
Erythrocyte sedimentation rate (mean $\pm$ SD) (mm/hr)	48.1 $\pm$ 26.3	43.2 $\pm$ 27.8	0.55
C-reactive protein (mean $\pm$ SD) (mg/dl)	64.3 $\pm$ 83.3	54.4 $\pm$ 82.7	0.70
Type of last implant operation (no. [%])			0.08
Primary	5 (28)	20 (56)	
Revision	13 (72)	16 (44)	
Last implant insertion before culture			
No. of months between the last implant operation and sonicate fluid culture (median [interquartile range])	34 (12.9–60.9)	23 (10.6–51.4)	0.70
Microbiologic test			
Synovial fluid culture (no./total no.)	8/18	10/33	0.31
Periprosthetic tissue culture (no. [%])			
≥ 1 positive culture	13 (72.2)	23 (63.9)	0.15
≥ 2 positive cultures	13 (72.2)	18 (50.0)	
Sonicate fluid culture (no. [%])			
Negative	4 (22.2)	15 (41.7)	0.23
Positive	14 (77.8)	21 (58.3)	

ultrasound to dislodge adherent bacteria. It has been found that *in vitro* application of pulsed ultrasound (46.5 kHz and 167 mW/cm²) can lead to an increase in the release of antimicrobials from antimicrobial-loaded cement or beads (6, 11), and low-frequency pulsed ultrasound (300 or 600 mW/cm²) has been found to result in an increase in the activity of antimicrobials against biofilm bacteria (the bioacoustic effect) (12), suggesting that antimicrobial-loaded PMMA could negatively impact sonicate culture results. The results of our study show that antimicrobial-loaded PMMA does not have an obvious negative impact on the microbiologic yield from explanted prostheses when sonication is used. In our study, there was a wide range of times between implantation and explantation (from 1 to 168 months). When we stratified the time interval, culture yield did not differ among the cases with or without antimicrobial-loaded PMMA (data not shown). In the literature, the com-

monly used antimicrobial doses range from 2.4 to 4.6 g of tobramycin and 1 to 4 g of vancomycin per 40 g of cement (3). In this study, the maximum dose was 2 g of vancomycin and 6 g of tobramycin. Although there are many unknowns associated with the addition of antimicrobials to PMMA, such as the biological activity when mixed with PMMA and how long the antimicrobial will release from PMMA *in vivo*, our study demonstrates that sonicate fluid culture can be applied irrespective of the inclusion of antimicrobials in PMMA.

There were a few limitations to this study. The sample size was small; because our institution is a referral center, whether antimicrobial-impregnated PMMA was used was unknown for a large number of the patients. Second, we did not measure antimicrobial concentrations in the sonicate fluid. Third, at our institution, cement is used more commonly in knee than in hip arthroplasties; therefore, there was a significant difference in the sites of arthro-

TABLE 2 Univariate analysis of risk factors associated with sonicate fluid culture positivity

Variable	Sonicate fluid culture–positive prosthesis (<i>n</i> = 35) (%)	Sonicate fluid culture–negative prosthesis (<i>n</i> = 19) (%)	<i>P</i> value
Patients demographics			
Age (mean ± SD) (yr)	67.1 ± 12.8	65.0 ± 16.2	0.60
Males (no. [%])	23 (66)	11 (58)	0.57
Site of arthroplasty (no. [%])			0.42
Knee	15 (43)	6 (32)	
Hip	20 (57)	13 (68)	
Risk factors for prosthetic joint infection (no. [%])			
Diabetes mellitus	5 (14)	2 (11)	>0.99
Immunosuppressive agents (steroids, methotrexate)	5 (14)	0	0.15
Visible purulence (no./total no.)	32/35	12/19	0.02
Acute inflammation in tissue (no./total no.)	21/25	12/16	0.69
Presence of sinus tract (no./total no.)	3/35	1/19	>0.99
Preoperative laboratory data			
Hemoglobin (mean ± SD) (mg/dl)	12.0 ± 1.3	11.7 ± 2.5	0.58
Blood leukocyte count (mean ± SD) (per mm ³)	9,690 ± 4,923	7,580 ± 2,285	0.04
Erythrocyte sedimentation rate (mean ± SD) (mm/hr)	50.4 ± 27.9	34.1 ± 22.9	0.04
C-reactive protein (mean ± SD) (mg/dl)	74.7 ± 95.3	24.4 ± 26.4	0.007
Type of last implant operation (no. [%])			
Primary	17 (49)	8 (42)	0.65
Revision	18 (51)	11 (58)	
Antimicrobial-loaded bone cement	14 (40)	4 (21)	0.23
Last implant insertion before culture (no. of months between the last implant operation and sonicate culture (median [interquartile range]))	25 (14.0–48.0)	30 (9.5–63.0)	0.98

TABLE 3 Microorganisms detected in 54 patients with prosthetic joint infection in this study

Type of cement	No. of patients	Microorganisms detected by sonicate fluid culture (no.)
Cement plus vancomycin and aminoglycoside	11	<i>Staphylococcus aureus</i> (3) CoNS ^a (2) <i>Peptostreptococcus magnus</i> (1) <i>Propionibacterium acnes</i> (1) <i>Proteus mirabilis</i> (1) <i>Corynebacterium striatum</i> (1) No growth (2)
Cement plus vancomycin	2	<i>S. aureus</i> plus CoNS ^a (1) No growth (1)
Cement plus gentamicin	2	<i>Streptococcus mitis</i> (1) No growth (1)
Cement plus tobramycin	3	CoNS ^a (3)
Cement with no antimicrobial	16	CoNS ^a (2) <i>S. aureus</i> plus CoNS ^a (1) <i>Staphylococcus aureus</i> (1) Viridans <i>Streptococcus</i> spp. (1) Group C <i>Streptococcus</i> spp. (1) <i>Haemophilus parainfluenzae</i> (1) <i>Corynebacterium striatum</i> (1) No growth (8)
Uncemented	20	CoNS ^a (6) <i>Staphylococcus aureus</i> (3) <i>Streptococcus agalactiae</i> (1) <i>Enterococcus faecalis</i> (1) <i>Propionibacterium avidum</i> (1) <i>Haemophilus parainfluenzae</i> (1) No growth (7)

^a CoNS, coagulase-negative *Staphylococcus* species.

plasty between the two study groups. Last, we studied only hip and knee implants, but we have no reason to believe that our findings would not apply to other types of prosthetic joints.

In conclusion, antimicrobial-loaded PMMA did not obviously influence the microbiologic culture yield assayed using sonicate fluid culture.

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