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# Is sonication of antibiotic-loaded cement spacers useful in two-stage revision of prosthetic joint infection?

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ABSTRACT

*Background:* In a two-stage exchange protocol for prosthetic joint infection (PJI), bacteria surviving over the antibiotic-loaded cement spacers may cause the persistence of infection with renewed clinical symptoms following the surgery. Culture after sonication of removed prosthesis is more sensitive than conventional periprosthetic tissue culture for the microbiological diagnosis of PJI. The aim of this study was to assess whether sonication of the spacer at the time of the second-stage procedure may improve the diagnosis of persistent PJI.
*Methods:* We evaluated by microbiological culture the sonication fluid from 222 cement spacers implanted in a two-stage exchange protocol in 157 patients affected by PJI. A mean of 1.3 (range, 1–4) spacer per patient was performed.
*Results:* In 53 out of 222 spacers analyzed infection was confirmed according to the MSIS criteria.

In 22 cases the infection was confirmed by both cultures on periprosthetic tissue and on sonication fluid from the spacers. In 23 cases persistent PJI was identified because of only cultures of periprosthetic tissue and 8 because of results obtained after spacer sonication.

The sensitivity of periprosthetic tissue cultures was higher than that of cultures performed on sonication fluid (84.9% vs 56.6%, $p < .001$).
*Conclusions:* Even though sonication of cement spacers has performances inferior than those reported for prosthesis, it can be considered a complementary method to unravel persistent infection during a two-stage exchange protocol for PJI.

## 1. Introduction

Two-stage prosthesis substitution is the standard treatment in chronic prosthetic joint infection (PJI) in knee and hip arthroplasty (Kunutsor et al., 2016). It entails removal of the prosthesis with debridement of all infected tissue and placement of an antibiotic-impregnated polymethylmethacrylate (PMMA) spacer, followed by broad spectrum antimicrobial therapy, and subsequent delayed reimplantation of a second prosthesis. The antimicrobial therapy must of course be adapted to a pathogen driven scheme as soon as the microbiological diagnosis becomes available. The spacer enables local delivery of antibiotic, preserves joint space and prevents the retraction soft tissues (Kurd et al., 2010).

High rates of satisfactory outcomes in terms of infection eradication have been reported using the two-stage exchange procedure. However, this approach is not uniformly successful (Nelson et al., 2014; Zalavras, 2014; Sorli et al., 2012; Mariconda et al., 2013; Schmolders et al., 2014). The presence of biofilm-forming pathogens is likely the leading cause for the infection to persist and, despite an initial high local antimicrobial discharge, the antibiotic-loaded cement itself, once the release of antibacterial molecules has ended, can represent an optimal biomaterial surface to which bacteria adhere, grow and possibly develop antibiotic resistance as a biofilm bacterial community (Neut et al., 2001).

Relapse of infection may be more frequent when drug resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Coagulase negative Staphylococci* (CoNS) are involved (Kurd et al., 2010; Mittal et al., 2007). The recurrence of PJI may be seen even with negative synovial fluid and intraoperative periprosthetic tissue cultures at the time of reimplantation (Berbari et al., 2007; Trampuz et al., 2007). Such clinical scenarios suggest the inadequacy of our current tests to identify a persistent subclinical infection. Given that microorganisms associated with a periprosthetic joint infection may adhere to a prosthesis surface through biofilm

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formation, an improved detection strategy such as sonication of the spacer may be useful (Trampuz et al., 2007; Sambri et al., 2018). Culture of samples obtained by sonication of the removed prosthesis has been shown to be more sensitive than conventional tissue cultures (Trampuz et al., 2007; Portillo et al., 2014; Puig-Verdie et al., 2013). However, only a few small-scale series have investigated the use of sonication of cement spacers (Nelson et al., 2014; Sorli et al., 2012; Mariconda et al., 2013).

In this study we investigated if the sonication treatment could be useful to identify bacteria from antibiotic-loaded cement spacers removed at the second surgical stage during a two-stage exchange procedure. Moreover, the patients were followed up in order to verify whether the risk of reinfection correlated to the microbiologic findings at the time of prosthesis reimplantation.

## 2. Material and methods

Between June 2014 and July 2016, 253 patients underwent two-stage revision surgery at our Institution (Istituto Ortopedico Rizzoli, Bologna, Italy) for suspected PJI of the knee or the hip. Among these, 157 patients had a clinically confirmed infection according to criteria defined by the Musculoskeletal Infection Society (MSIS) (Parvizi et al., 2013) and were included in the present study (Fig. 1). The study was approved by our institution ethics committee. Informed consent was obtained from all patients enrolled.

Patients' characteristics are reported in detail in Table 1.

The average time between prosthesis implantation and the first stage of two-stage procedure for PJI was 28 months (range, 2–336 months). At surgery, the infected prosthesis was removed and sent for sonication to the microbiology laboratory, followed by an extensive surgical debridement, irrigation and antibiotic-loaded (with gentamicin) PMMA spacer implantation (Donati and Biscaglia, 1998; Anagnostakos, 2017).

Empirical intravenous antibiotic therapy was started intraoperatively, after at least five tissue specimens were taken from representative areas within the effective joint space. All patients were discussed at the multidisciplinary infection board and the antibiotic therapy was given according to antibiotic sensitivity test (AST) whenever available.

After a minimum of 6 weeks from the first stage and after an "antibiotic holiday" of 2 weeks, if there was still clinical evidence of persistent infection, the patient underwent repeat debridement and spacer exchange and the antibiotic therapy was maintained. If no signs of infection were observed and laboratory results did not suggest residual infection, the patient underwent the second stage of the prosthetic revision (prosthesis implantation) in the absence of antimicrobial treatment.

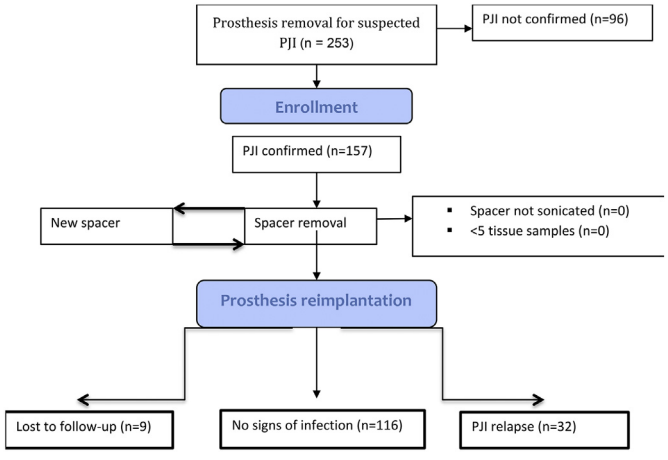


**Fig. 1.** The flowchart shows enrolment and follow-up of the patients. According to clinical and laboratory presentation, 253 patients had prosthesis removed for a suspected prosthesis joint infection (PJI). Of these, 157 had PJI confirmed according to the Musculoskeletal Infection Society (MSIS) criteria and were included in the study.
Nine patients were lost to follow-up.
At final follow-up, 32 patients had a PJI relapse.

**Table 1**
Patients' characteristics at baseline.

| Patients characteristic | ($n$ = 157) |
|---|---|
| Sex | 93 female/64 male |
| Mean age (range), years | 63 (21–93) |
| Mean ESR (range), mm/h[a] | 22 (2–72) |
| Mean CRP (range), mg/dl[a] | 1.2 (0.1–17.0) |
| Joints prosthesis | 55 hip/102 knee |

[a] At the time of the second stage intervention

At the time of cement-spacer removal, the spacer was removed in the operating room under sterile conditions and transported to the microbiology laboratory for sonication. Five separate biopsies from the periprosthetic tissues were collected. In all the patients this specificy diagnostic protocol was followed and none of them were excluded from the study population. Homogenization of tissue samples was performed in the original container, vortexing the specimen in 3 mL tryptic soy broth. Each tissue specimen homogenate was inoculated in sheep blood agar, thioglycollate broth medium, and tryptic soy broth. All the media were incubated at 36 ± 1 °C for 7 days and examined daily for evidence of growth. For isolation of individual colonies, aliquots from enrichment broth tubes were spread using a sterile loop on Columbia CNA blood agar, mannitol salt agar, MacConkey agar, and chocolate agar and incubated at 36° ± 1 °C under aerobic conditions for 24 h. They also were subcultured on chocolate agar under anaerobic conditions for 72 h at 36° ± 1 °C. All the media were from Biolife Italiana (Milan, Italy). Negative thioglycollate broth medium and tryptic soy broth incubates were reincubated up to 14 days at 36° ± 1 °C and examined daily for evidence of growth. Identification and antimicrobial susceptibility testing were performed with a MicroScan® WalkAway® system (Beckman Coulter, Sacramento, CA, USA) (Sambri et al., 2018).

Homogenization of tissue samples was performed in the original container, vortexing the specimen in 3 mL tryptic soy broth. Each tissue specimen homogenate was inoculated in sheep blood agar, thioglycollate broth medium, and tryptic soy broth. All the media were incubated at 36 ± 1 °C for 7 days and examined daily for evidence of growth. For isolation of individual colonies, aliquots from enrichment broth tubes were spread using a sterile loop on Columbia CNA blood agar, mannitol salt agar, MacConkey agar, and chocolate agar and incubated at 36° ± 1 °C under aerobic conditions for 24 h. They also were subcultured.

on chocolate agar under anaerobic conditions for 72 h at 36° ± 1 °C. All the media were from Biolife Italiana (Milan, Italy). Negative thioglycollate broth medium and tryptic soy broth incubates were reincubated up to 14 days at 36° ± 1 °C and examined daily for evidence of growth. Identification and antimicrobial susceptibility testing were performed with a MicroScan® WalkAway® system (Beckman Coulter, Sacramento, CA, USA) (Trampuz et al., 2007).

Sonication was performed according to the technique of Trampuz et al. (Berbari et al., 2007). Briefly, the container was filled with sterile saline until the device was submersed, carefully sealed, vortexed, and sonicated in an ultrasound bath (VWR International Srl, Milan, Italy) for 5 min with a frequency of 40 kHz at room temperature. At the end of sonication, the obtained fluids were collected in sterile tubes and centrifuged at 3000 rpm for 10 min at room temperature. The pellet was suspended in a volume of 2 mL of the same solution. A total of 100 mL of each sample was plated on chocolate agar, sheep blood agar, and inoculated in tryptic soy broth and thioglycollate broth medium. Sheep blood agar was incubated aerobically, whereas chocolate agar plates

were incubated in 5% $CO_2$ atmosphere at 36° ± 1 °C for 7 days. Broths were incubated for 7 and 14 days at36° ± 1 °C and, if negative, the incubation was extended to 14 days and examined daily for evidence of growth; terminal.

subcultures were performed. Subcultures and identification were performed as previously described for the cultures performed on biopsy specimen. Sonication fluids were considered positive if at least five colonies grew on agar plates after 24 h and up to 7 days or if growth was observed during broth enrichment.

After the microbiologic testing, persistent infection of the spacer was defined according to the MSIS criteria (Parvizi et al., 2014). Results obtained from cultures performed on sonicated fluid were considered together with those from cultures performed on tissue samples (Sambri et al., 2018). Tissue and sonication fluid cultures were scored as positive when at least one pathogen was identified (*Staphylococcus aureus, Pseudomonas aeruginosa and Enterobacteriaceae)*, or when two separated cultures yielded a skin commensal organism (*coagulase negative staphylococci-CoNS or Propionibacterium acnes*). (Osmon et al., 2013; Societe de Pathologie Infectieuse de Langue F, 2010) The results obtained with cultures of tissue samples and sonication fluids were compared.

Successful eradication of the infection was defined as the absence of clinical and/or laboratory evidence of infection at 6 months after the second stage or at latest follow-up.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of tissue and sonication fluids cultures were calculated and compared. Differences between the results obtained were assessed with the McNemar test. Probability values $< 0.05$ were considered significant. All analyses were completed using the Statistical Package for Social Science (SPSS Statistics for Windows, Version 22.0; IBM Corporation, Armonk, NY, USA).

## 3. Results

A mean of 1.3 (range, 1–4) spacer per patient was performed. Therefore, a total of 222 spacers were removed and analyzed. The mean in-situ period of each spacer was 111 days (range, 45–173).

In 53 out of the 222 spacers analyzed, the infection was confirmed according to the MSIS criteria. Among these infected spacers, in 48 cases the same pathogen that had caused the initial infection was isolated.

Overall, cultures on periprosthetic tissue and on sonicated fluids from the spacers yielded concordant results in 186 (83.8%) out of 222 cases. In details, 22/186 (11.8%) cases of infection were confirmed by cultures from both periprosthetic tissues and fluids from sonication. In 164/186 (88.2%) cases both types of samples gave negative cultures. In 36 cases results of tissue and sonication cultures were discordant. More specifically, 5 cases of infection were not confirmed according to the MSIS criteria, while the remaining 31 patients had persistent infection. Of these 31, 23 were identified according to the results of cultures from periprosthetic tissue and 8 only from cultures performed on sonication fluids from spacers.

The most commonly isolated pathogens were Gram positive pathogens (20 strains of CoNS of which 10 were methicillin-resistant and 10 methicillin-sensitive). The remaining isolated strains included 2 methicillin-sensitive *Staphylococcus aureus* (MSSA, 3 isolates of *Enterococcus* spp. in three and *Pseudomonas* aeruginosa was found in the last 4 patients.

The sensitivity of cultures performed on periprosthetic tissues were significantly higher than the value detected for cultures from sonication fluids.

The specificity, positive predictive value (PPV) and negative predictive value (NPV) did not differ between tissue and sonication fluids cultures (Table 2).

Nine patients were lost during the follow-up period.

At final follow-up (average 18 months, range 7–44) 109 patients had neither clinical nor laboratory signs of infection. Thirty-two patients (20.3%) had a PJI relapse. Twenty-three of these (71.8%) had positive cultures (either on tissue specimen or sonication fluid) at the time of reimplantation. A higher incidence of relapsing PJI was observed in patients with a multiple spacer exchange due to the persistent infection than those in which a single spacer was used ($p = .021$).

## 4. Discussion

The two-stage exchange is the standard in the treatment for PJI. High frequency of success has been reported with this strategy, with an eradication rate of both total knee arthroplasty (Jamsen et al., 2009) and total hip arthroplasty (Senthi et al., 2011) infection of > 80%. Despite the adoption of the two-stage exchange protocol, there are still cases of persisting PJI where the cement spacer itself may act as a biomaterial surface that facilitates the survival and proliferation of microorganisms. (Neut et al., 2001) Hence, it is of paramount importance to actively look for bacterial biofilm on the removed cement spacer to determine whether the infection has been efficiently and completely removed.

Surprisingly, we found a higher sensitivity of cultures performed on periprosthetic tissues over cultures of sonicated fluids. These data differs from the results obtained previously with smaller patients series. (Nelson et al., 2014; Mariconda et al., 2013) In the present study, all spacers were removed after an “antibiotic holiday” of 2 weeks and this might explain the higher performances of cultures from tissue samples than previously reported (Portillo et al., 2014; Puig-Verdie et al., 2013). Moreover, the quite high antibiotic concentrations released from the cement spacer during sonication can be high enough to cause false-negative results of cultures on spacer sonication fluid. (Mariaux et al., 2018)

In addition, the very high diagnostic performances of cultures performed on periprosthetic tissue samples might be explained by the fact that a total of 5 specimens are currently harvested from each patient. It was reported that the sensitivity of tissue cultures increases according to the number of samples evaluated (Trampuz et al., 2007).

The sensitivity of cultures from sonication fluids from cement spacers looks quite irrelevant for diagnostic purposes. Nevertheless, the diagnosis of persistent infections was possible in 8 additional cases by combining the use of cultures from sonicated fluids and periprosthetic tissue specimens at second-stage surgery. This is indeed potentially relevant finding, as subclinical infection is frequently associated with a poor clinical outcome and the precise identification of the real status of each infection plays a major role in the long-term prognosis of these patients. In our opinion, sonication of cement spacers can be considered an additional tool in the diagnostic workflow of persistent PJI during the two-stage procedure. According to this statement the results obtained from cultures performed on sonicated fluid from spacer could be considered when analyzing the MSIS criteria to diagnose PJI.

We found that those patients who underwent more than one second-stage surgery due to persistent infection had a higher risk of PJI recurrence. This might be relevant in the choice of the prosthesis at the time of reimplantation, as for example a knee arthrodesis can reduce the risk of reinfection. (Gottfriedsen et al., 2016) Moreover, the early identification of the pathogens involved in persistent infection after reimplantation may help in giving pathogen specific suppressive antibiotic therapy as soon as possible after surgery. (Siqueira et al., 2015)

Selected cases of reinfection were caused by microorganisms that were different from those involved in the primary original infection. This is not surprising being a different etiology a common finding reported in many studies evaluating the outcomes after management of periprosthetic joint infections (Kurd et al., 2010; Mittal et al., 2007; Haleem et al., 2004). It is therefore possible that some reinfections resulted from of a novel pathogen introduction (Rakow et al., 2018) rather than from a persistency of bacteria not originally detected by standard intraoperative culture testing. A complex and prolonged

**Table 2**
Performance of the diagnostic techniques.

| Cultures type | Tissue cultures | Sonication fluid cultures | P[a] |
|---|---|---|---|
| Sensitivity [95% C.I.] | 84.9% (45/53) [77.2–92.5] | 56.6% (30/53) [48.8–63.2] | < 0.001 |
| Specificity [95% C.I.] | 99.4% (168/169) [95.4–100] | 97.6% (165/169) [93.6–100] | 0.824 |
| Positive predictive value (PPV) | 97.8% (45/46) | 88.2% (30/34) | 0.187 |
| Negative predictive value (NPV) | 95.5% (168/176) | 87.7% (165/188) | 0.161 |

[a] McNemar test

surgery in patients undergoing multiple procedures is indeed associated with a higher infection rate than for primary procedure.

Some limitations of this study must be addressed. At first this is a study based on a retrospective cases series, with its inherent limitations. In addition, cultures from both periprosthetic tissues and from sonication fluids of the spacers were included within the MSIS criteria for infection, which in this study was used as the gold standard for the presence or absence of infection and that do not presently specific how to manage the results of the cultures used in this study from spacers. As a consequence the lack of definite criteria to state the presence of re-infection or complete cure is a potential criticism of the present and every study evaluating PJI. Therefore, we used the MSIS criteria as the gold standard for confirming the presence or absence of PJI even at the stage of spacer removal (Parvizi et al., 2014). Furthermore, the lack of longer-term clinical data did not allow us to confirm whether each single patient with excluded PJI according to the MSIS criteria was later developing a clinical infection. However, we don't think that a long-term follow-up could be helpful in confirming real aseptic patients since a late infection could also be absent at the time of the study but this could develop later on.

In conclusion, the performances of cultures from sonication fluids of cement spacers are lower than those reported for prosthesis (sensitivity 56.6% vs 89.0%) (Sambri et al., 2018). However, this procedure can be considered as a complementary method that could help to unravel the possible persistence of microorganisms on an antibiotic-loaded cement spacer during a two-stages exchange protocol. The use of this technique in addition to cultures on periprosthetic tissues may help to confirm whether an infection has been definitely cleared, or whether further therapeutic options, such as the early debridement of the revision prosthesis, are necessary.

## References


Anagnostakos, K., 2017. Therapeutic use of antibiotic-loaded bone cement in the treatment of hip and knee joint infections. J. Bone Joint Infect. 2, 29–37.

Berbari, E.F., et al., 2007. Culture-negative prosthetic joint infection. Clin. Infect. Dis. 45, 1113–1119.

Donati, D., Biscaglia, R., 1998. The use of antibiotic-impregnated cement in infected reconstructions after resection for bone tumours. J. Bone Joint Surgery Br. Vol. 80, 1045–1050.

Gottfriedsen, T.B., et al., 2016. Knee arthrodesis after failure of knee arthroplasty: a nationwide register-based study. J. Bone Joint Surg. Am. 98, 1370–1377.

Haleem, A.A., et al., 2004. Mid-term to long-term followup of two-stage reimplantation for infected total knee arthroplasty. Clin. Orthop. Relat. Res. 35–39.

Jamsen, E., et al., 2009. Outcome of prosthesis exchange for infected knee arthroplasty: the effect of treatment approach. Acta Orthop. 80, 67–77.

Kunutsor, S.K., et al., 2016. Re-infection outcomes following one- and two-stage surgical revision of infected knee prosthesis: a systematic review and meta-analysis. PLoS One 11, e0151537.

Kurd, M.F., et al., 2010. Two-stage exchange knee arthroplasty: does resistance of the infecting organism influence the outcome? Clin. Orthop. Relat. Res. 468, 2060–2066.

Mariaux, S., et al., 2018. Diagnosis of persistent infection in prosthetic two-stage exchange: evaluation of the effect of sonication on antibiotic release from bone cement spacers. J. Bone Joint Infect. 3, 37–42.

Mariconda, M., et al., 2013. Sonication of antibiotic-loaded cement spacers in a two-stage revision protocol for infected joint arthroplasty. BMC Musculoskelet. Disord. 14, 193.

Mittal, Y., et al., 2007. Two-stage reimplantation for periprosthetic knee infection involving resistant organisms. J. Bone Joint Surg. Am. 89, 1227–1231.

Nelson, C.L., et al., 2014. Sonication of antibiotic spacers predicts failure during two-stage revision for prosthetic knee and hip infections. Clin. Orthop. Relat. Res. 472, 2208–2214.

Neut, D., et al., 2001. Biomaterial-associated infection of gentamicin-loaded PMMA beads in orthopaedic revision surgery. J. Antimicrob. Chemother. 47, 885–891.

Osmon, D.R., et al., 2013. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin. Infect. Dis. 56, e1–e25.

Parvizi, J., et al., 2013. Proceedings of the International Consensus on Periprosthetic Joint Infection. Bone Joint J. 95-B, 1450–1452.

Parvizi, J., et al., 2014. Definition of periprosthetic joint infection. J. Arthroplast. 29, 1331.

Portillo, M.E., et al., 2014. Advantages of sonication fluid culture for the diagnosis of prosthetic joint infection. J. Infect. 69, 35–41.

Puig-Verdie, L., et al., 2013. Implant sonication increases the diagnostic accuracy of infection in patients with delayed, but not early, orthopaedic implant failure. Bone Joint J. 95-B, 244–249.

Rakow, A., et al., 2018 Oct 26. Origin and characteristics of haematogenous periprosthetic joint infection. Clin. Microbiol. Infect (in press).

Sambri, A., et al., 2018. Is treatment with dithiothreitol more effective than sonication for the diagnosis of prosthetic joint infection? Clin. Orthop. Relat. Res. 476, 137–145.

Schmolders, J., et al., 2014. Evidence of MRSE on a gentamicin and vancomycin impregnated polymethyl-methacrylate (PMMA) bone cement spacer after two-stage exchange arthroplasty due to periprosthetic joint infection of the knee. BMC Infect. Dis. 14, 144.

Senthi, S., et al., 2011. Infection in total hip replacement: meta-analysis. Int. Orthop. 35, 253–260.

Siqueira, M.B., et al., 2015. Chronic suppression of periprosthetic joint infections with oral antibiotics increases infection-free survivorship. J. Bone Joint Surg. Am. 97, 1220–1232.

Societe de Pathologie Infectieuse de Langue F, et al., 2010. Recommendations for bone and joint prosthetic device infections in clinical practice (prosthesis, implants, osteosynthesis). Societe de Pathologie Infectieuse de Langue Francaise. Med. Mal. Infect. 40, 185–211.

Sorli, L., et al., 2012. The relationship between microbiology results in the second of a two-stage exchange procedure using cement spacers and the outcome after revision total joint replacement for infection: the use of sonication to aid bacteriological analysis. J. Bone Joint Surgery Br. Vol. 94, 249–253.

Trampuz, A., et al., 2007. Sonication of removed hip and knee prostheses for diagnosis of infection. N. Engl. J. Med. 357, 654–663.

Zalavras, C.G., 2014. CORR Insights(R): Sonication of antibiotic spacers predicts failure during two-stage revision for prosthetic knee and hip infections. Clin. Orthop. Relat. Res. 472, 2215–2216.