

# Superiority of the sonication method against conventional periprosthetic tissue cultures for diagnosis of prosthetic joint infections

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## Abstract

**Introduction** Diagnosis of periprosthetic infections is challenging. The aim of this study was to compare the diagnostic accuracy of conventional periprosthetic tissue culture and culture of sonication fluid of the explanted prostheses.

**Methods** We prospectively enrolled 114 patients undergoing revision hip or knee arthroplasty because of loosening of the prostheses, at our institution, between July 2012 and July 2016. Patients' medical history and demographic characteristics were recorded. The explanted hardware was separated in sterile containers and sonicated under sterile conditions. At least five samples of periprosthetic tissue

were sent for culture and histological examination. We compared the culture of samples obtained by sonication of explanted hip and knee prostheses with conventional culture of periprosthetic tissue for the microbiological diagnosis of prosthetic joint infection.

**Results** Infectious Diseases Society of America guidelines were used for the definition of prosthetic joint infection. Sixty-one patients had periprosthetic infection and 53 aseptic loosening (73 hip prostheses and 41 knee prostheses). The sensitivity of sonication fluid culture was 77.04%, and the sensitivity of conventional tissue cultures was 55.73% ( $p$  value = 0.012). The specificities of the two methods were 98.11 and 94.34%, respectively. The sensitivity of the histopathological examination of the periprosthetic tissue was 72.10%. There were 17 patients with PJI where the isolated pathogen was detected in SFC but not in PTC, while in five cases the pathogen was detected only in PTC. There were nine patients where no bacteria were detected by any microbiological method and the diagnosis was based on clinical and histological findings, according to the guidelines.

**Conclusions** The sonication method represents a reliable test for the diagnosis of prosthetic joint infections with a greater sensitivity and specificity than the conventional periprosthetic tissue cultures.

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**Keywords** Sonication · Biofilm · Periprosthetic infections · Total joint arthroplasty

## Introduction

Total hip and knee arthroplasties are two of the most successful orthopedic surgical operations, relieving chronic pain and improving quality of life. Periprosthetic joint

infection (PJI) is the most devastating complication of total joint arthroplasty, associated with high morbidity, prolonged hospitalization and substantial cost [1]. The expected increasing number of arthroplasties because of demographic changes will unavoidably lead to an increase in PJIs, in the next decades [2].

Differentiation of PJI from aseptic loosening remains a challenging diagnostic problem. As the application of anti-bacterial drugs is of paramount importance in the treatment of PJI, the isolation and identification of the responsible microorganism are pivotal. PJI is considered to be associated with the presence of bacterial glycocalyx biofilms attached to the implant, where the bacteria have changed their phenotypes to an extremely tough form of life with an increased antibiotic resistance [3]. Moreover, bacteria of the biofilm enter a stationary state in which they are less susceptible to growth-dependent antibiotics [4]. Conventionally, the periprosthetic tissue culture (PTC) is the gold standard in the microbiological diagnosis of PJI. However, due to previous use of antibiotics and the formation of biofilm which protects bacteria from detection and elimination, the sensitivity of conventional culture methods does not exceed 75% [3, 5]. Thus, the disaggregation of the biofilm from the surface of the explanted prostheses enables a sampling of vital bacteria, improving the results of the culture and the outcome of the treatment of the infection.

Sonication is one of the most promising methods for the diagnosis of PJI. The diagnostic value of the application of long-wave ultrasound before cultures for the disruption of the prostheses biofilm and the enhancement of the bacterial growth has been introduced by Trampuz et al. in 2007 [6]. The method dislodges the adherent bacteria, providing a significant better recovery of bacterial growth in culture than the conventional culture of periprosthetic tissue samples for the microbiological diagnosis of PJIs. The purpose of this prospective study was to compare the diagnostic accuracy of conventional periprosthetic tissue culture and culture of fluid derived from vortexing and bath sonication of the explanted total hip and knee prostheses, evaluating the usefulness of sonication fluid culture (SFC) in the diagnosis of PJI.

## Materials and methods

### Patient selection

Between July 2012 and July 2016, we investigated 114 patients, 29 men and 85 women, undergoing revision hip or knee arthroplasty because of loosening of the prostheses, at our hospital. Seventy-three patients had undergone total hip replacement and 41 total knee replacements. Preoperative measures of C-reactive protein (CRP) and erythrocyte

sedimentation rates (ESR) were obtained in all subjects. The explanted hardware was separated in sterile containers and sonicated under sterile conditions. At least five samples of periprosthetic tissue were sent for culture and histological examination. Demographic characteristics, clinical, laboratory and microbiological data of the patients were recorded. The study design was assessed and approved by the institutional review board before the beginning of the study.

### Patient eligibility

For the definition of PJI, Infectious Diseases Society of America (IDSA) guidelines were used [7]. According to these guidelines, one of the following criteria is definitive evidence of PJI: (a) presence of sinus tract that communicates with the prosthesis, (b) presence of acute inflammation on the histopathological examination of the periprosthetic tissue, (c) presence of visible purulence surrounding the prosthesis and (d) two or more positive intraoperative PTCs or positive SFC. Aseptic failure was defined as loosening of the prosthesis in the absence of any the above criteria. SFC was considered positive when it yielded >50 colony-forming units (CFU)/ml of the same organism. Isolation of a bacterial species in a single tissue sample or yielding <50 CFU/ml in sonication fluid was classified as false positive. PJIs were classified according to the onset of symptoms, as early, delayed and late (less than 3 months, 3–24 months, more than 24 months, respectively) [8]. Inflammation in the histopathological examination was defined as >5 neutrophils per high-power field. Previous antimicrobial therapy was defined as administration of antimicrobial agents during the 14 days before removal of the prostheses.

### Periprosthetic tissue cultures

For all patients, at least five intraoperative periprosthetic tissue specimens were collected from the bone–cement/bone–prosthesis interface, from capsule and from soft tissues with obvious inflammatory changes. Tissue specimens were located into sterile boxes and individually homogenized in 3 ml Trypticase soy broth for 1 min using mortar and pestle. Tissue homogenate samples were inoculated in 0.1 ml aliquots onto aerobic (SBA) and anaerobic sheep blood agar (ASBA) plates and in 1 ml aliquots into thioglycollate broth. The cultures were incubated at 37 °C for 10 days. A terminal subculture was performed from all thioglycollate broth specimens on blood agar plates and incubated at 37 °C for 5 more days. Positive tissue cultures were considered those with the same microorganism isolation of at least two periprosthetic tissue samples. Each unique colony of isolated microorganisms was identified,

and their antimicrobial susceptibility was tested by using standard automatic methods (Vitek-2 system; bio Mérieux, Marcy L'Etoile, France). Two more periprosthetic tissue samples were sent for histological examination.

### Sonication fluid cultures

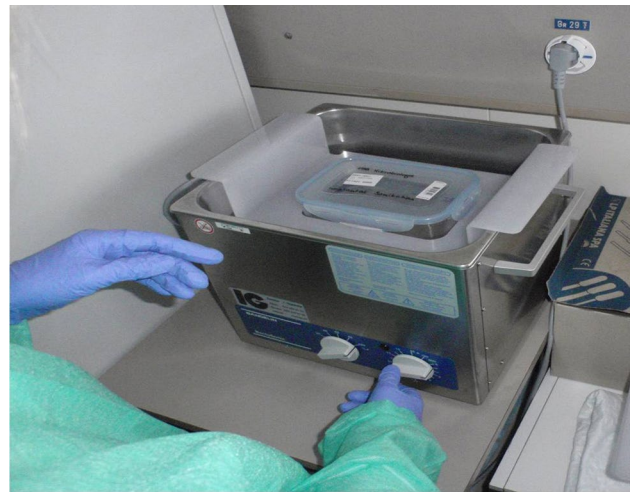
The explanted prosthesis (or its components) was aseptically removed in the operating room and located in a wide-mouthed, sterile, solid airtight container (Lock & Lock; Vertrag AG, Stafa, Switzerland). The implant was transported to the microbiology laboratory and sonicated within 6 h, inside an ultrasound bath (Figs. 1, 2). Sonication of the implant was performed according to the method described by Trampuz et al. [6]. Aliquots of 0.1 ml sonicate fluid were inoculated onto sheep blood agar (SBA) and anaerobic sheep blood agar (ASBA) plates. Additionally, 1 ml of the remaining of sonication fluid was added in 10 ml thioglycollate broth (TGB). The SBA plates and TSB were incubated at 37 °C aerobically and the ASBA plates and TGB at 37 °C anaerobically and inspected daily for bacterial growth.

### Statistical analysis

The sensitivities, specificities, positive predictive values and negative predictive values of the different methods were calculated with two-by-two contingency tables.



**Fig. 1** Sterile container in ultrasound bath



**Fig. 2** Sonication process in ultrasound bath

Ninety-five percent confidence intervals (95% CI) were calculated as exact binomial confidence intervals. The sensitivity of the different culture methods was compared by McNemar's test of paired proportions. A probability  $p$  value  $<0.05$  was considered statistically significant. Statistical analysis was performed using the PASW 18 (SPSS release 18.0; SPSS Inc., Chicago, Illinois).

### Results

During the study period, 114 patients who were operated because of loosening of total hip and knee arthroplasty were enrolled. Sixty-one patients were revised because of septic loosening and 53 because of aseptic loosening. Demographics, reason for arthroplasty, age of implants, preoperative and perioperative findings are shown in Table 1. The mean age of the patients was 70 years (range 45–88 years). Primary osteoarthritis was the most common cause for both total hip and knee arthroplasty. The mean time from implantation to revision or resection surgery was longer in patients with aseptic loosening than in patients with periprosthetic infection. There were 8 early PJI, 17 delayed PJI and 36 late PJI. No patient received any previous antimicrobial therapy during the 14 days before removal of the prostheses.

Table 2 demonstrates the sensitivity of the culture of sonication fluid versus the culture of periprosthetic tissue in different study groups. The overall sensitivity of SFC was 77.05% (95% CI 0.65–0.87), and the sensitivity of PTC was 55.74% (95% CI 0.42–0.68,  $p$  value = 0.01). Moreover, SFC had a higher specificity (98.11%) in comparison with PTC (94.34%). The sensitivity of the histopathological examination of the periprosthetic tissue was 72.10%.

**Table 1** Characteristics of study subjects

Characteristics	Subjects with aseptic loosening ( <i>n</i> = 53)	Subjects with PJI ( <i>n</i> = 61)
Mean age (years)	70 (range 45–85)	70 (range 49–88)
Sex		
Male	15 (28.3%)	14 (22.9%)
Female	38 (71.7%)	47 (77.1%)
Reason for arthroplasty		
Primary osteoarthritis	38 (71.7%)	47 (77.1%)
Inflammatory joint disorder	3 (5.7%)	3 (4.9%)
Fracture/trauma	7 (13.2%)	9 (14.7%)
Congenital abnormalities	4 (7.5%)	2 (3.3%)
Avascular necrosis	1 (1.9%)	0
Site of arthroplasty		
Hip	34 (64.2%)	39 (63.9%)
Knee	19 (35.8%)	22 (36.1%)
Mean age of implant (months)	115 (range 1–336)	71 (range 1–432)
Hip	135 (range 1–336)	83 (range 1–432)
Knee	78 (range 5–240)	52 (range 2–228)
Perioperative findings		
Sinus tract	0	17 (27.8%)
Visible purulence	0	27 (44.2%)
Positive cultures	0	34 (55.7%)
Tissue inflammation	0	44 (72.1%)
Preoperative findings		
Serum CRP > 1 mg/dl	24 (45.3%)	33 (54.1%)
ESR > 30 mm/hr	17 (32.1%)	40 (65.6%)

**Table 2** Sensitivity of the culture of the sonication fluid versus the culture of periprosthetic tissue

	Sensitivity of sonication fluid culture (%)	Sensitivity of periprosthetic tissue culture (%)	<i>p</i> value
Overall	77.04	55.73	<b>0.01</b>
Type of infection			
Early	87.5	62.5	0.48
Delayed	64.7	41.1	0.13
Late	80.6	61.1	0.14
Site of infection			
Hip	84.6	58.9	<b>0.02</b>
Knee	63.6	50.0	0.45

Statistical significance for *p*-value < 0.05

As shown in Table 2, sensitivity of SFC is superior to the sensitivity of PTC in all study groups, with a statistical significance in periprosthetic hip infections (*p* value = 0.02). Table 3 compares specificity, positive predictive value and negative predictive value between the two methods. There was one case of aseptic loosening with a false-positive SFC that yielded 10 CFU/ml considered as contamination by a coagulase-negative staphylococcus. Respectively, we observed three cases with a false-positive PTC, where bacteria were detected in only one sample of periprosthetic tissue. There were 17 patients with PJI where the isolated pathogen was detected in SFC but not in PTC, while in five cases the pathogen was detected only in PTC. There were nine patients where no bacteria were detected by any

**Table 3** Specificity, positive predictive value and negative predictive value of the culture of the sonication fluid versus the culture of periprosthetic tissue

	Sonication fluid culture		Periprosthetic tissue culture	
	Value (%)	95% CI	Value (%)	95% CI
Specificity	98.11	89.9–99.9%	94.34	84.3–98.8%
Positive predictive value	97.92	87.0–99.7%	91.89	78.7–97.2%
Negative predictive value	78.79	70.1–85.5%	64.94	58.1–71.2%

microbiological method and the diagnosis was based on clinical and histological findings, according to IDSA guidelines. In cases of infection, *coagulase-negative staphylococci* were isolated in 42.3% of patients; *staphylococcus aureus* was isolated in 13.5% of patients, Gram-negative bacteria in 32.7% of patients and other bacteria in 11.5% of patients. PTC was positive in 65.7% of Gram (+) infections and in 64.7% of Gram (–) infections, while the corresponding rates for SFC were 85.7 and 94.1%, respectively.

## Discussion

PJI is one of the most demanding complications in reconstructive hip and knee surgery. Early and reliable diagnosis is important, as it allows selection of the best antimicrobial therapy. Understanding the etiology of infection is significant. In PJIs, bacteria have the capacity to tightly adhere to artificial surfaces, forming protective biofilms, which make them resistant to antibiotics and difficult to detect with conventional tissue cultures [6, 9]. Dapunt et al. [10] have supported the theory that the presence of biofilm elicits an inflammatory response which triggers the generation of bone-resorbing osteoclasts, leading to osteolysis and loosening. Classical culture methods often fail to detect the pathogen, and considerable effort has been focused on developing alternative approaches of bacteria identification. Sonication dislodges these bacteria from the prosthesis allowing them to be cultured, without disturbing their viability [6]. In the present study, we compared the results of microbiological cultures from sonication fluid with classical microbiological cultures of periprosthetic tissues.

The results of our study demonstrate that in our group of hip and knee PJIs, SFC had an overall sensitivity of 77.04% in comparison with 55.73% sensitivity of PTC. This difference was found to be statistically significant ( $p$  value = 0.012). In 17 patients, the diagnosis of PJI was based only on the SFC, as no bacteria were detected on the PTC, supporting the hypothesis that sonication increases the chance of bacteria isolation in culture-negative PJIs. Our results are in agreement with the study by Trampuz et al. [6] with similar numbers of reported sensitivity for SFC (78.5%) and PTC (60.8%). Furthermore, the higher sensitivity of sonication method, ranging from 67 to 91%, as opposed to tissue cultures, has been confirmed by many previous studies [11–19]. Two recent meta-analyses showed that SFC is of great value for the diagnosis of PJI with a greater sensitivity than PTC (79%), especially for patients with previous antibiotic treatment [20, 21]. On the contrary, Kempthorne et al. [22] have found that SFC is less sensitive than PTC, but the lack of use of histopathological examination may have underestimated the true incidence of PJI.

In our study, no patient received antibiotics for at least 14 days prior to revision surgery. In the large prospective study of Trampuz et al. [6] including 331 cases, the sensitivity of SFC was statistically significant over standard tissue cultures in cases where antibiotics have been administered within 14 days from revision surgery. A further study of 112 PJIs also commented on the negative effect of recent antibiotic administration on the sensitivity of PTC but not on the sensitivity of the SFC [23]. Scorzolini et al. [18] found that the sensitivity of SFC was not affected by the timing of antibiotic interruption before surgery. On the contrary, the study by Puig-Verdie et al. [9] of 317 PJIs did not confirm these findings; nevertheless, the authors acknowledged that only 19 patients had antibiotics within 14 days from revision surgery.

Our methodology aimed to ensure maximum sensitivity of both SFC and PTC. At least five tissue specimens were collected intraoperatively as this has been shown to increase the sensitivity of PTC at its maximum [6, 24]. Before sonication, the specimens were vortexed for 30 s. Vortexing has been shown to increase the positive cultures following sonication of prosthesis [6, 25]. Vortexing alone has been shown to have an inferior result as opposed to vortexing and subsequent sonication [26, 27]. All the prostheses were sonicated within solid containers in order to avoid the possibility of contamination as shown in studies where plastic bags have been used. In our study, we performed standard culture of the sonication fluid. Moreover, the high cutoff value of 50 CFU/ml for the SFC was set to avoid false-positive results due to contamination.

Sonication with subsequent polymerase chain reaction (PCR) of the cultured fluid has been shown to have improved results [15, 28]. A further study demonstrated that combination of sonication and PCR can identify microorganisms in presumed aseptic loosening with negative PTC [29]. Nevertheless, the widely reported range of specificity of this technique has raised concerns as to its broad application [24]. We feel that, at present, there is no definitive evidence for the routine use of PCR of the sonicate fluid of hip and knee prostheses. A few studies have been published, reporting that the use of dithiothreitol is more sensitive than the sonication method, but its diagnostic value needs to be further investigated [30–32].

In our study, SFC demonstrated a better sensitivity of 80.6% in patients with late infections as opposed to 61.1% of PTC. In agreement is a recent study of 317 PJIs where SFC was found to have higher diagnostic accuracy in patients with late infections. The theory behind this is that in acute infections the microorganisms have not formed biofilms as yet, and therefore, tissue culture has also good sensitivity [9]. Nevertheless, in our study, SFC demonstrated higher sensitivity in early infections as well (87.5%) as opposed to PTCs (62.5%). However,

these differences in sensitivity are not statistically significant. In 2013, the International Consensus Meeting on Periprosthetic Joint Infection advocated against the routine sonication of explanted prosthesis. The group concluded that SFC should be used in cases of suspected or proven prosthetic joint infections in which preoperative aspirates have failed to reveal any pathogens and in cases where antibiotics have been administered within 2 weeks from revision surgery [24]. The study by Puig-Verdie et al. [9] declared that the SFC is recommended only in delayed implant failures. On the other hand, other published studies have come to conclusion that the sonication method is quite reliable and sufficient for pathogen detection in the clinical diagnostic routine [33–35]. We believe that the use of sonication is a cheap and useful test, with high sensitivity in all patient subcategories and should be applied as a routine in PJI investigation.

Table 2 shows statistically significant superiority of the SFC in periprosthetic hip infections but not in knee infections. A possible explanation would be the wider use of antibiotic-loaded cement in total knee arthroplasties, which could affect the structure of the biofilm, decreasing the sensitivity of SFC. Moreover, the absolute number of periprosthetic knee infections in this study is relatively low, reducing the possibility of statistically significant results.

Limitations of our study include the relatively small number of patients as well as that only hip and knee arthroplasties were included. No molecular diagnostic method, such as PCR analysis of the sonicate fluid, was used to confirm bacterial isolation. Furthermore, none of the patients received antibiotics within 2 weeks from surgery which did not give us the opportunity to assess the effect of this parameter in the comparison between sonicate fluid and tissue cultures.

The results of our study demonstrate that SFC has a statistically higher sensitivity than PTC for PJIs around knee and hip arthroplasties. The technique is simple and can be performed in most microbiological laboratories. Taking into consideration that in patients with PJIs the tissue cultures are occasionally negative, we advocate the broad use of sonication as a particularly valuable diagnostic tool. Our findings need to be confirmed in a larger patient series and in other joint arthroplasties.

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**Compliance with ethical standards**

**Conflict of interest** All authors declare that they have no conflict of interest.

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