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ORIGINAL ARTICLE

Sonication of removed implants improves microbiological diagnosis of postoperative spinal infections

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Abstract

Purpose In total joint replacement, culturing of the sonication fluid of removed implants has proven to be more sensitive than conventional periprosthetic tissue culture for the microbiological diagnosis of prosthetic joint infection. However, its role in postoperative spinal implant infection (PSII) is not well investigated. Therefore, the aim of this study was to determine the validity of sonication in detecting infection following instrumented spine surgery.

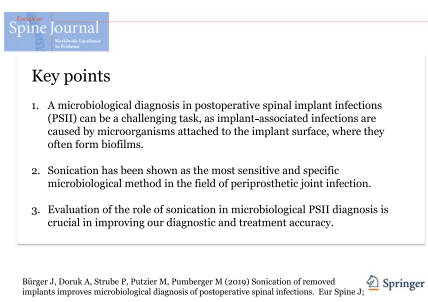
Methods In this prospective controlled consecutive cohort study, patients undergoing spinal revision between September 2016 and March 2018 were analyzed. In all patients sonication of removed spinal implants and at least one peri-implant tissue culture were performed. Demographic data, including age, gender, clinical manifestation, comorbidities, laboratory values (CRP and blood leukocytes), were recorded. Microorganisms causing PSII were documented. Sensitivity and specificity of sonication and peri-implant tissue culture were evaluated.

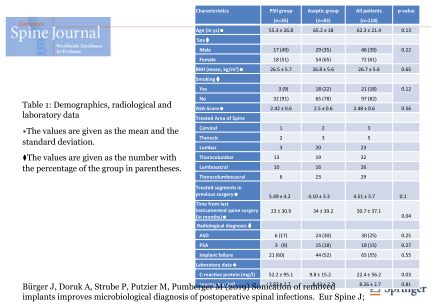
Results A total of 118 patients were included. PSII was diagnosed in 35 patients, representing 29.6% of the study cohort. Sensitivities of tissue and sonication fluid culture were 65.7% (95% confidence interval (CI) 48.6–80.0) and 94.3% (95% CI 85.7–100) (p value = 0.002) and specificities 96.4% (95% CI 91.6–100) and 98.8% (95% CI 96.4–100), respectively. The most common microorganisms found in PSII were coagulase-negative Staphylococci and *Propionibacterium acnes*. Eleven PSIIs were detected only by sonicate fluid culture.

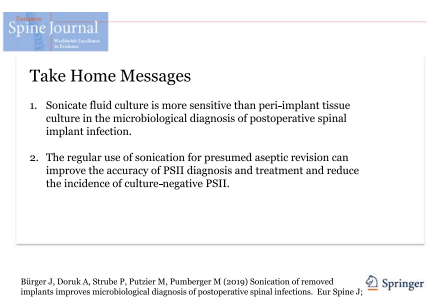
Conclusions Culture of samples obtained by spinal implant sonication was more sensitive than conventional peri-implant tissue culture for the microbiological diagnosis of PSII. Therefore, sonication should be used as a routine tool in the diagnostic workup of PSII.

Graphical abstract

These slides can be retrieved under Electronic Supplementary Material.







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Extended author information available on the last page of the article

Keywords Sonication · Infection · Diagnosis · Spine · Low virulence

Introduction

The number of spine surgeries performed worldwide is increasing over the last years [1, 2]. Although new techniques and minimal invasive procedures are developing, the incidence of postoperative spinal implant infections (PSII) is reported as up to 20% in the literature [3, 4]. Infections after spine surgery lead to longer hospitalization, higher mortality and morbidity, resulting with increased costs to healthcare system [5]. Furthermore, the implantation of spinal instrumentation is associated with a higher risk of developing a postoperative infection than implant-free surgical interventions due to possible attachment of bacteria on the avital implant surface [6]. However, a microbiological diagnosis in these cases can be a challenging task due to the presence of microorganisms in biofilms attached to the implant surface, which cannot be sampled by peri-implant tissue [7]. This condition led to culture analysis of specimens obtained from the explanted devices. With the use of sonication, it is possible to detach bacteria from their biofilm and make them accessible for further culture analysis [8]. Culturing of sonication fluid specimens has been shown to be more sensitive than conventional tissue cultures in the field of periprosthetic joint infection [8–10]. However, little data are available for the use of sonication in diagnosing PSII. Previously, Sampedro et al. [11] showed implant sonication followed by culture to be more accurate than peri-implant tissue culture for the diagnosis of PSII, with a sensitivity and specificity of 91% and 97%, respectively. To our knowledge, this is the only study investigating the role of sonication in the diagnosis of PSII in the literature so far. Therefore, the aim of this study was to determine the role of sonication in the diagnosis of PSII and compare our results to the results of Sampedro et al.

Materials and methods

In this prospective study, patients who underwent revision surgery after instrumented spinal surgery at our institution between September 2016 and March 2018 with one or more peri-implant tissue specimen, as well as spinal implants (rods, screws) submitted for sonication, were analyzed. Removed implants were collected by a no-touch technique and were sent to the microbiology laboratory within 1 h and processed immediately. Patients were excluded, if retained implants were possibly contaminated by skin contact. A quality assessment tool was developed on the basis of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement and used to assess reported study quality [12]. The study protocol was reviewed and approved by the institutional ethics committee and was done in accordance with the Declaration of Helsinki.

Postoperative spinal implant infection was defined, if ≥ 1 of the following criteria was met: (1) intraoperative purulence surrounding the implant, (2) sinus tract communicating with the implant, (3) acute inflammation or peri-implant membrane Type II or III by Morawietz [13] in histopathological examination of peri-implant tissue or (4) positive tissue or sonication culture as described below. Patients not meeting these criteria were classified as aseptic failure.

There are four conditions, where peri-implant tissue culture was considered as positive: first, a detection of low-virulent microorganisms (coagulase-negative Staphylococci (CNS), Propionibacteria, *Bacillus* spp., *Finegoldia magna*, *Enterococcus* spp.) in at least two specimens; second, a detection of low-virulent microorganisms in at least one specimen, if patient was under antimicrobial treatment in last 30 days prior surgery; third, a detection of low-virulent microorganisms in one specimen, confirmed by the same microbial growth in sonicate fluid culture; and fourth, a detection of high-virulent microorganisms (*Staphylococcus aureus*, *Streptococcus* spp., *Enterobacteria*) in at least one specimen. Otherwise, the sample was classified as negative.

Sonication was performed using a BactoSonic 14.2 unit (Bandelin, Berlin, Germany). We used the same technique as described by Janz et al. [8]. Sonication culture was classified positive in case of detection of > 50 colony-forming units (CFU) of low-virulent bacteria, detection of < 50 CFU of low-virulent bacteria with previous antimicrobial treatment in the last 30 days, detection of < 50 CFU of low-virulent bacteria with the detection of the same organism in peri-implant tissue culture or detection of high-virulent bacteria of any amount. The growth of > 50 CFU of Micrococcus luteus or Ralstonia pickettii was classified as contamination.

Clinical signs such as fever, pus or sinus tract and radiographic signs and laboratory markers (C-reactive protein (CRP) and leukocytes), as well as the number of treated segments, were analyzed.

Descriptive summaries for septic and aseptic group were analyzed by using Chi-squared or Fisher's exact test. The two-sample *t* test (for parametric distribution) or Mann–Whitney *U* test (for nonparametric distribution) was used to compare continuous variables between groups. Sensitivity and specificity of sonication and peri-implant tissue culture were calculated with 95% exact binomial confidence interval. For comparison between sonication and peri-implant tissue culture, McNemar's test was used. For all tests a p value of < 0.05 was considered as significant.

SPSS version 20 (SPSS Inc., Chicago, Illinois) was used for the statistical analyses.

Results

We identified 141 patients, of which 23 were excluded, 4 due to obvious contamination and 19 due to the absence of microbiological samples. Of the remaining 118 patients analyzed, 80 (68%) had histopathological examination. PSII was diagnosed in 35 patients, representing 29.6% of the study cohort.

Totally, 639 segments, 217 in PSII group and 422 in aseptic group, were treated. Most common reason for revision surgery in both groups was implant failure followed by wound healing problem in infection group and adjacent segment degeneration (ASD) in aseptic group. PSII occurred significantly more frequent within 1 year after the last instrumented spine surgery than aseptic failure (57.1% vs. 24.1%, p value = 0.018). The mean time interval between current and last instrumented spine surgery was 23 months for PSII group and 32.5 months for aseptic group (p value = 0.15). Demographics, clinical and radiological signs and laboratory data are shown in Table 1. Sensitivities of tissue and sonication fluid culture were 65.7% (95% confidence interval (CI) 48.6–80.0) and 94.3% (95% CI 85.7–100) (p value = 0.002) and specificities 96.4% (95% CI 91.6–100) and 98.8% (95% CI 96.4–100), respectively. One PSII was detected by tissue but not sonicate fluid culture, whereas 11 PSIIs were detected only by sonicate fluid culture. Four of these 11 patients had an antimicrobial treatment within last 30 days prior to surgery. Only in one PSII, sonicate fluid and peri-implant tissue culture were negative and the diagnosis was made by positive histopathological result. A combination of sonicate fluid and peri-implant tissue culture showed a sensitivity of 97.1%. Eleven patients received antimicrobial treatment within the last 30 days prior surgery, and seven of them had PSII. Sensitivities of peri-implant tissue and sonicate fluid culture in these cases were 57.1% and 100%, respectively.

Table 1 Demographics, radiological and laboratory data

Characteristics	PSII group (n = 35)	Aseptic group (n = 83)	All patients (n = 118)	p value
Age (in years)[a]	55.3 ± 26.8	65.2 ± 18	62.3 ± 21.4	0.13
Sex[b]				
Male	17 (49)	29 (35)	46 (39)	0.22
Female	18 (51)	54 (65)	72 (61)	
BMI (mean, kg/m^2)[a]	26.5 ± 5.7	26.8 ± 5.6	26.7 ± 5.6	0.65
Smoking[b]				
Yes	3 (9)	18 (22)	21 (18)	0.12
No	32 (91)	65 (78)	97 (82)	
ASA score (median, range)	2 (1–4)	3 (1–4)	2(1–4)	0.46
Treated area of spine				
Cervical	1	2	3	
Thoracic	2	3	5	
Lumbar	3	20	23	
Thoracolumbar	13	19	32	
Lumbosacral	10	16	26	
Thoracolumbosacral	6	23	29	
Treated segments in previous surgery[a]	5.49 ± 4.2	4.10 ± 3.3	4.51 ± 3.7	0.1
Time from last instrumented spine surgery (in months)[a]	23 ± 30.9	34 ± 39.2	30.7 ± 37.1	0.04
Radiological diagnosis[b]				
ASD	6 (17)	24 (30)	30 (25)	0.25
PSA	3 (9)	15 (18)	18 (15)	0.27
Implant failure	21 (60)	44 (52)	65 (55)	0.55
Laboratory data[a]				
C-reactive protein (mg/l)	52.2 ± 95.1	9.8 ± 15.2	22.4 ± 56.2	0.03
Leukocytes (/nl)	7.87 ± 2.7	8.43 ± 2.7	8.26 ± 2.7	0.81

[a]The values are given as the mean and the standard deviation

[b]The values are given as the number with the percentage of the group in parentheses

The most common microorganisms found in PSII were CNS, especially *Staphylococcus epidermidis*, and *P. acnes*. CNS were found in 16, and *P. acnes* in 9 patients with PSII. *Staphylococcus aureus* was the causative microorganism in two patients, of which one was methicillin-resistant. In 22 patients the same microorganism was isolated in both peri-implant tissue and sonicate fluid culture. Table 2 summarizes the causative pathogens in PSII patients.

Discussion

This current study showed that sonicate fluid culture is more sensitive than peri-implant tissue culture in the microbiological diagnosis of PSII. Our results are concordant with the results of Sampedro et al. and support already described superiority of sonicate fluid culture in the field of periprosthetic joint infection. In an attempt to further improve the diagnostic accuracy, we combined the sonicate fluid and peri-implant tissue culture. This represented the highest achieved sensitivity of 97%. When regarding only

Table 2 Detected microorganisms in cases of PSII

Case°	Only found in peri-implant tissue	Concordant	Only found in sonicate fluid
1	*Enterobacter cloacae* complex	–	–
2	*Bacillus firmus*	*E. coli*	–
3	*E. coli*	*S. epidermidis*, *Peptoniphilus asaccharolyticus*	*Finegoldia magna*
4	–	*P. acnes*	–
5	–	*P. acnes*	–
6	–	*P. acnes*	–
7	–	*P. acnes*	–
8	–	*P. acnes*	–
9	–	*P. acnes*	–
10	–	*P. acnes*	–
11	–	*S. epidermidis*	–
12	–	*S. epidermidis*	–
13	–	*S. epidermidis*	–
14	–	*S. epidermidis*	–
15	–	*S. epidermidis*	–
16	–	*S. schleiferi*	–
17	–	*S. warneri*	–
18	–	*Enterobacter cloacae* complex	–
19	–	*E. coli*	–
20	–	*Enterococcus faecalis*	–
21	–	*S. haemolyticus*, *S. capitis*	–
22	–	*S. epidermidis*	*Enterococcus faecalis*
23	–	*Pseudomonas aeruginosa*, *Candida albicans*, *Candida krusei*	MRSA, *Enterococcus faecalis*
24	–	–	*P. acnes*
25	–	–	*P. acnes*
26	–	–	*S. epidermidis*
27	–	–	*S. epidermidis*
28	–	–	*S. epidermidis*
29	–	–	*S. epidermidis*
30	–	–	*S. epidermidis*
31	–	–	*S. epidermidis*
32	–	–	*S. lugdunensis*
33	–	–	*Streptococcus parasanguinis*
34	–	–	*S. aureus*, *Finegoldia magna*
35	–	–	–

the microbiological methods, it was possible to identify the causative microorganism in eleven additional patients through the use of sonicate fluid culture, while peri-implant tissue culture was negative. This can be explained by biofilm-forming properties of the surface-adhering microorganisms. Non-planktonic microorganisms do not replicate readily in laboratory culture and can easily escape detection by conventional culture-based methods [14]. Lack of confirmation of a causative microorganism poses a challenge with regard to the selection of an appropriate antimicrobial and surgical treatment. The regular use of sonication for presumed aseptic revision therefore can improve the accuracy of PSII diagnosis and treatment and reduce the incidence of culture-negative PSII.

Furthermore, a recent study by Romano et al. highlighted the potential economic advantage to hospitals associated with the routine use of antibiofilm techniques for microbiological diagnosis of periprosthetic joint infection [15]. After pooling direct and indirect costs associated with false positive and negative results of the different diagnostic techniques, sonication has been shown to be increasingly cost-effective due to extra cost generated by diagnostic inaccuracy of traditional tissue cultures in 20% or more patients compared to 2% with sonication. Thus, the potential benefits of sonication in more accurate pathogen identification can lead to an improvement in the management of implant-related infections with a substantial economic balance or advantage [15].

Although the intraoperative cultures remain the definite indicator of the infection presence [6], other diagnostic tools such as laboratory or imaging studies are still useful for diagnosing PSII in the preoperative setting. C-reactive protein (CRP) has been shown to be an accurate marker for diagnosing especially acute PSII with high sensitivity and specificity [16]. Magnetic resonance imaging (MRI) can be a very useful study to diagnose PSII. Gadolinium enhancement improves the diagnostic accuracy of MRI and should be used if infection is suspected [6]. However, a recent study suggested that 18F-FDG PET/CT should be the first-line cross-sectional imaging study in patients with suspected spinal hardware infection due to accurate diagnosis of non-infectious hardware complications and alternative sources of infection, including pneumonia [17].

Interestingly, low-virulent microorganisms such as CNS and Propionibacteria were the most isolated causative microorganisms in our study cohort. The high incidence of low-virulent microorganisms was described previously in many studies reporting about delayed PSII [11, 18–20] and is consistent with the general concept that peri-implant infections arise from native skin flora [20, 21]. There remains much debate regarding the role of *Propionibacterium acnes* as contaminant or infectious microorganisms in implant-associated infections. However, *Propionibacterium acnes* has been recently identified as a common cause of late-presenting infection after spinal surgery [19, 22, 23]. A study by Jakab et al. [24] documented clearly the pathogenic potential of Propionibacteria in causing late postoperative infections. Shifflett et al. [20] described also high incidence of *Propionibacterium acnes* in patients (54%) undergoing revision surgery due to pseudarthrosis (PSA). Previous studies dealing with *Propionibacterium acnes* infection in periprosthetic joints reported that multiple (≥ 2) positive cultures are indicative of true infection [25, 26]. Allowing one positive culture to define PSII may be prone to misdiagnose patients, who are aseptic, as having PSII (false positive), leading to unnecessary surgical and antimicrobial treatment. In our study 3 of 18 patients (16.7%) with the diagnosis of PSA had positive cultures and *P. acnes* was isolated in 2 of them. By comparison, highly virulent species such as Staph. aureus were only present in 5% of the study cohort. This can be explained by the different management strategies in early- and late-onset infections, since early infections can be best managed with debridement and implant retention and late infections with implant removal [23]. Therefore, we could mostly include patients with late-onset infections, which are more typically caused by low-virulent microorganisms [18].

Previous antimicrobial treatment can reduce the accuracy of tissue culture and is therefore associated with poor microbiological culture results [27]. Contrary to the findings of Sampedro et al. and other studies [9, 11, 27], in the present study sensitivity of sonicate fluid culture increased up to 100% in patients receiving preoperative antimicrobial treatment. This could be explained by the low number of patients with preoperative antimicrobial treatment in our study.

Interestingly, we identified significant more patients treated ≥ 5 segments in PSII compared to aseptic group (p value $= 0.01$). Pereira et al. and Abdul-Jabbar et al. also described the treatment of ≥ 3 segments as a risk factor for spinal infections [21, 28]. Surgical approaches for many segments provoke more soft tissue damage and increase the risk of dead space formation [29]. These factors could result in a poor vascular perfusion and tissue necrosis leading to local immunodeficiency, offering microorganisms an optimal environment to proliferate.

This study has some limitations. The sample size was small and therefore can be underpowered. Furthermore, patients with only one tissue culture were not excluded from the study, which can alter our results. However, every patient had sonication fluid culture and most of them also histopathological results making a reliable diagnosis of PSII possible. There is no gold standard definition for PSII leading to different incidence in each study causing non-comparable results. The impact of sonication duration, incubation time and cutoff value for CFU for the diagnosis of an infection is not well established. A major problem of sonication is the risk of contamination, and the doubtfulness

in the interpretation of positive results in a single sample. So, a multimodal approach including clinical, paraclinical, microbiological and histopathological findings is necessary for the diagnosis of PSII.

In conclusion, sonication fluid culture is more sensitive than peri-implant tissue culture for the microbiological diagnosis of PSII and can reduce the incidence of culture-negative infections. Therefore, we recommend using sonication as a routine tool in the diagnostic workup of PSII.

Compliance with ethical standards

Conflict of interest No conflicts of interest for the current study.

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