Evaluation of sonicate fluid cultures in comparison to histological analysis of the periprosthetic membrane for the detection of periprosthetic joint infection

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Abstract

Purpose The aim of this prospective study was to evaluate the diagnostic efficacy of sonicate fluid cultures (SFC) and the histological analysis of the periprosthetic membrane (PM) for the detection of periprosthetic joint infection (PJI).

Methods The histological samples were evaluated according to the consensus classification of PM as defined by Morawietz and Krenn. All explanted endoprosthesis were subject to sonication. Additionally, a synovial aspiration and microbiological culture of tissue samples were performed for each patient. Twenty three of the 59 patients had an established PJI.

Results Sonication achieved the highest sensitivity out of all diagnostic methods with 91 % and a specificity of 81 %. The PM achieved a sensitivity of 87 % and a specificity of 100 %. In three cases of PJI a pathogen was isolated solely by sonication while all other microbiological methods were negative. In seven cases there was a positive bacterial culture through sonication with negative histology.

Conclusions Our results show a high correlation between the microbiological and histological results. In our patient group sonication achieved the highest sensitivity out of all diagnostic methods and was more sensitive than conventional microbiological methods.

Introduction

When performing revision surgery of a loosened endoprosthesis it is essential to exclude a septic cause. PJI are difficult to diagnose because they are often missing the classic symptoms of infection such as fever, erythema and wound discharge. The cause for this absence of systemic symptoms is that the causative pathogen is primarily present in a sessile form and isolated within a biofilm. It is because of this lack of planktonic bacteria that the isolation of vital bacteria for a microbiological diagnosis is difficult [4, 8]. Several studies have shown that the histological diagnosis of PJI has a superior sensitivity compared to the microbiological diagnosis [3, 6, 7, 9]. A widely accepted and clinically practical classification system for a histological diagnosis of PJI is the classification of the periprosthetic membrane (PM) according to Morawietz and Krenn [5]. While the advantage of a histological diagnosis is the high sensitivity, the main disadvantage is that the causative bacterial specimen remains unknown. This leads to a therapeutic dilemma of initiating an antibiotic treatment without knowing the causative pathogen. The second disadvantage of a diagnosis of PJI based solely on histology is that there is a discordance between the histological and microbiological results in about 10 % of all cases [5]. While the interobserver reproducibility of the PM is sufficiently high at 85 %, this also means that it is not infallible. Sonication has been proven to be the most sensitive and specific out of all microbiological methods [10, 11]. Through sonication it is possible to dislodge sessile bacteria from their biofilm and make them accessible for culture [12]. This enables detection of PJI which would not be possible with other microbiological

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methods, such as synovial aspiration or culture of tissue samples. We hypothesise that SFC achieve a higher sensitivity in diagnosing PJI than the PM.

Patient selection and methods

Patient selection

Fifty-nine patients who underwent a revision or explantation surgery of a total hip or knee arthroplasty at our institution from October 2010 until March 2011 were selected for participation in this prospective study. To be eligible for inclusion all patients underwent a complete or partial prosthesis exchange or a complete implant removal. Additionally, a routine preoperative laboratory screening including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and multiple samples of periprosthetic tissue were taken from the area macroscopically most indicative of infection. A minimum of two samples were obtained from the vicinity of each explanted component. Patients where a sampling of the PM was not possible were excluded from this study. The study design, patient protocols, and consent forms were approved by our local ethics committee before commencement of this study. To be eligible for participation all patients had to give their written consent, be over 18 years of age, and not have received antibiotics for a minimum of 14 days prior to surgery. Thirty-three female and 26 male patients were included and the average patient age was 67 years. Forty one of the 59 patients received a prosthesis or component exchange for aseptic loosening and 18 patients underwent prosthesis explantation because of preoperative suspicion for PJI. All patients with a preoperative suspicion of PJI had a planned two-stage exchange.

Diagnosis of infection

A preoperative PJI was defined according to the following criteria: intra-articular presence of pus or a sinus tract, a PM indicative of infection (infectious type II or combined type III) [11], or a positive microbiological culture in one of the following two samples, a preoperative intra-articular aspiration, which was additionally confirmed by a positive culture of intraoperative tissues, or a positive culture of the same bacterial species in a minimum of two different tissue samples [10]. Paraclinical parameters such as the ESR, leucocytes and CRP were excluded from our criteria for PJI. However since the determination of CRP is part of the clinical routine, it was included in our study. If none of the above-mentioned parameters were present a PJI was excluded.

Preoperative joint aspiration

A preoperative joint aspiration was performed for all patients. All antibiotics were discontinued for at least 14 days prior to aspiration. The aspiration was performed according to the national guidelines for intra-articular aspiration in an operating theatre with laminar airflow. The aspiration site was disinfected three times, covered in sterile drapes and a skin incision was made prior to insertion of the aspiration cannula. The intraarticular position of the cannula was confirmed by image intensifier and all samples were cultured both aerobically and anaerobically for 14 days.

Intraoperative sampling

An intraoperative sampling of periprosthetic tissue was performed from the area which was macroscopically most indicative of infection in the immediate vicinity of the explanted component. Between two and five periprosthetic tissue samples were obtained for each patient. If multiple components were removed tissue samples were acquired from the vicinity of each component. Additionally a complete removal of the PM was performed. If the explanted components came into contact with the patient's skin during explantation the component was excluded from this study. The sterile polypropylene containers were opened in the operating theatre immediately before component explantation. The components were placed directly in the containers and submerged in sterile Ringer's solution. The containers were hermetically sealed and stored at room temperature until sonication was performed. Sonication was performed within six hours of explantation and the microbiological samples were transported to the Institute for Microbiology and Hygiene for culture and final analysis.

Sonication protocol

Sonication was performed using a BactoSonic® 14.2 (Bandelin, Berlin, Germany) ultrasonic unit. The tank fluid was mixed with contact liquid (Ticopur TR3, Bandelin, Berlin, Germany) to facilitate the conduction of ultrasound waves. The efficacy of the BactoSonic® 14.2 unit was tested at regular intervals with the manufacturer recommended foil test to ensure a thorough and uniform sonication process. Prior to sonication the samples were vortexed for 30 seconds. To facilitate the distribution of ultrasound waves the sealed containers were placed in polycarbonate trays and suspended over the tank floor. Sonication was performed for three minutes at an intensity of 100 %. After sonication the containers were briefly vortexed and 24 ml of sonicate fluid were sampled using a sterile syringe. The sonicate fluid was divided evenly between an anaerobic and aerobic blood culture bottle and a plastic

syringe. All samples were sent directly to our Institute for Microbiology for culture and final analysis. To monitor the risk of contamination negative controls consisting of empty polypropylene containers which were treated in the same manner as the explanted endoprosthesis were performed at regular intervals. None of these samples provided a positive microbiological culture.

Microbiological analysis

The SFC were analysed at the Institute of Microbiology and Hygiene, Charité, Berlin. The sonicate fluid was inoculated in various culture media (standard e.g., blood and chocolate agar, Brain-heart bouillon, Wilkins Chalgren agar, McConkey agar, Sabouraud agar and thioglycolate broth). All samples were cultured in the usual manner for 14 days to guarantee acquisition of slow growing species [8]. All other acquired microbiological samples such as periprosthetic tissue samples and synovial aspiration were cultured in the same manner.

Histological analysis

The tissue samples were obtained during surgery and immediately fixed in 4 % formalin. All samples were embedded in paraffin blocks and sliced into 5- μ m thick sections. The tissue sections were stained with haematoxylin and eosin. These slides were analysed under normal and polarized light microscopy. The PM was classified according to Morawietz and Krenn into type I (wear particle induced), type II (infectious type), type III (combined type), and type IV (indeterminate type) [5].

Statistical analysis

The presence of PJI was defined as previously stated. The sensitivity, specificity, positive and negative predictive values were calculated individually for the following diagnostic parameters: CRP, intraoperative tissue cultures, PM, and SFC (Table 1). Figure 1 shows the true positive and false positive results for detection of PJI.

The highest possible specificities of 100 % for PM and intraoperative tissue cultures can be explained through their inclusion in our criteria for PJI. All cases of a PM indicative of infection or positive intraoperative tissue samples were automatically defined as a PJI and resulted in a specificity of 100 % and negative predictive value of 100 %.

Results

The sensitivities, specificities, positive and negative predictive values for CRP, intraoperative tissue samples, PM, and SFC are shown in Table 1. In our study SFC achieved the highest sensitivity (91 %) out of all microbiological methods and also surpassed the histological classification of the PM (87 %).

Table 2 shows the results for the PM for all cases including the 23 cases of established PJI. Of the three cases (Pat. 6; 9; 25) of established PJI with a PM not indicative of infection (2x type I, 1x type IV), all three patients had positive intraoperative tissue cultures. Two of these three cases (Pat. 9;25) could also be confirmed by SFC.

Tables 3 and 4 display all cases of isolated bacteria and their corresponding classification of PM. The microbiological results are grouped by SFC and conventional microbiological methods.

Our results show a high concordance between the PM and SFC. Out of the 23 cases with a defined PJI 20 were identified as positive by histology and 21 cases were identified as positive by SFC (Fig. 1). Out of the 20 cases of PJI which were correctly identified by histology as indicative of infection 20 were confirmed by SFC while only 15 could only be correctly identified by intraoperative tissue cultures. When regarding only the microbiological methods, 18 of the 23 cases of PJI could be correctly identified by intraoperative tissue cultures and 21 could be identified by SFC.

In seven cases there was a positive microbiological culture solely by SFC with negative tissue cultures and negative histology. According to our definition of PJI these seven cases were classified as false positive and are not included in our 23 cases of defined PJI. The membranes classified in

Table 1 Sensitivity, specificity, PPV, and NPV for the individual and grouped parameters

Parameters	Sensitivity	Specificity	NPV	PPV
CRP	83 %	72 %	87 %	66 %
Intraoperative tissue cultures	75 %	100 %	86 %	100 %
PM	87 %	100 %	92 %	100 %
SFC	91 %	81 %	94 %	75 %
PM + SFC	96 %	77 %	96 %	76 %
PM + CRP	96 %	72 %	96 %	69 %
SFC + CRP	96 %	59 %	96 %	60 %

NPV negative predictive values, PPV positive predictive values, CRP C-reactive protein, PM periprosthetic membrane, SFC sonicate fluid cultures

Fig. 1 Accuracy of diagnosis of periprosthetic joint infection (PJI) for individual and groups of diagnostic methods

these cases were type I six times and type IV once. The isolated bacterial species in these cases were CNS six times and *Propionibacterium acnes* once.

In an attempt to improve the diagnostic significance, combinations of different diagnostic parameters (CRP and SFC, CRP and PM, and SFC and PM) were made and the results are displayed in Fig. 1.

Discussion

Several studies have been able to show the superiority of the histological diagnosis of PJI over microbiological methods [6, 9]. The aim of our study was to analyze whether SFC were able to surpass the sensitivity and specificity of the PM. In our study population SFC showed the highest sensitivity out of all individual parameters with 91 % for the diagnosis of PJI. The histological analysis of PM achieved the second highest sensitivity with 81 % (Table 1). This proves our hypothesis that SFC surpasses the sensitivity of the PM for diagnosis of PJI.

When regarding only the microbiological methods it was possible to isolate the causative bacterial species in three additional cases through the use of SFC than by culture of periprosthetic tissue alone. This can be explained by the improved sampling of SFC over PM. The closer the sampling is to the actual site of infection, in this case the implant–bone interface, the higher is the theoretical bacterial concentration which can be sampled and cultured. Multiple

intraoperative tissue samples were taken from the vicinity of all the explanted components. Since some patients received only a partial exchange of a single component, this resulted in the non-standardised number of acquired intraoperative tissue samples.

In an attempt to further improve the diagnostic sensitivity we formed a group comprised of SFC and PM. When the PM was regarded in combination with SFC 22 of the 23 cases of defined PJI could be positively identified. This represents the highest achieved sensitivity in our study of 96 %. The one case of PJI which was not detected was a revision of a loose acetabular component with no preoperative suspicion of PJI, but two positive intraoperative tissue cultures. During the further clinical course a septic failure ensued and in the following prosthesis explantation CNS were isolated in the intraoperative synovial aspiration, intraoperative tissue cultures and SFC.

Out of these 23 cases with a defined PJI it was possible to isolate a pathogen through intraoperative tissue cultures in 18 cases and through SFC in 21 cases. In two of the three cases of defined PJI, with a PM not indicative of infection, it was possible to isolate a causative pathogen through intraoperative tissue samples and additionally confirm the identical bacterial species (*Propionibacterium acnes* and *Staphylococcus epidermidis*) by SFC. The resulting histological classification was a type IV and a type I membrane, respectively. We can therefore conclude that the use of SFC in addition to intraoperative tissue cultures is a helpful tool to confirm bacterial infection in cases of false negative histology. The third case, which was classified as a type I membrane, could not be confirmed by PM or SFC. Preoperatively this case was diagnosed as an aseptic failure and a one stage exchange was performed. The positive microbiological cultures were acquired from intraoperative tissue cultures (*Staphylococcus epidermidis*). In accordance with the current recommendation for therapy of early PJI, a course of antibiotics was initiated for this patient [2]. In the further clinical course nine weeks after the exchange procedure, this patient presented clinical signs of a PJI and received a prosthesis explantation. During the explantation procedure a type III PM and the same bacterial species (*Staphylococcus epidermidis*) were isolated from the intraoperative synovial aspiration, intraoperative tissue cultures and SFC.

Table 2 Classification of PM for all cases, cases with no suspicion of infection, and cases of established infection

Classification of the PM	All cases	No suspicion of infection	Established infection
Type I (wear particle induced)	29	27	2
Type II (infectious type)	14	0	14
Type III (combined type)	6	0	6
Type IV (indeterminate type)	10	9	1

Table 3 Single bacterial species isolated through sonicate fluid culture

Patient	Sonicate fluid culture	Periprosthetic membrane	Intraoperative tissue samples and intraop. synovial aspiration
9	<i>Propionibacterium acnes</i>	Type I	<i>Propionibacterium acnes</i>
10	CNS	Type I	
14	CNS	Type I	
17	<i>Staph. aureus</i>	Type I	<i>Staph. aureus</i>
31	<i>Propionibacterium acnes</i>	Type I	
48	CNS	Type I	
56	CNS	Type I	
13	CNS	Type II	CNS
21	CNS	Type II	
23	MRSA	Type II	
29	<i>Propionibacterium acnes</i>	Type II	
30	<i>Staph. aureus</i>	Type II	<i>Staph. aureus</i>
36	CNS	Type II	CNS
41	<i>Staph. aureus</i>	Type II	<i>Staph. aureus</i>
45	MRSA	Type II	MRSA
47	CNS	Type II	CNS
27	<i>Streptococcus agalactiae</i>	Type III	<i>Strept. agalactiae</i>
35	CNS	Type III	CNS
46	CNS	Type III	CNS
50	<i>Propionibacterium acnes</i>	Type III	CNS

This constellation is most likely indicative of an intraoperative infection during revision surgery with an ensuing septic failure.

In seven patients it was possible to isolate bacteria solely from SFC. The PM was not indicative of infection in any of these patients. It is known that there is an intraobserver correlation coefficient of 85 % for the PM [5]. It is therefore not possible to say with complete certainty whether these

cases were defined as not indicative of infection because of a histological misclassification or if the isolated bacteria were false positive. According to our definition of PJI all cases of single positive SFC were classified as false positive. The clinical consequence of a positive SFC would be the postoperative initiation of specific antimicrobial therapy. When one considers the possible consequence of a false positive microbiological diagnosis, one must alternatively

Table 4 Multiple bacterial species isolated through sonicate fluid culture

Patient	Sonicate fluid culture	Periprosthetic membrane	Intraoperative tissue samples and intraop. synovial aspiration
24	CNS <i>Propionibacterium acnes</i>	Type I	2× CNS
40	2x CNS	Type I	
1	CNS <i>Staph. aureus</i>	Type II	
19	CNS <i>Staph. aureus</i> <i>Enterococcus faecalis</i>	Type II	CNS
42	CNS <i>Bacillus circulans</i>	Type II <i>Propionibacterium acnes</i>	CNS
32	CNS + <i>Staph. aureus</i> <i>Clostridium bifermentans</i>	Type III	CNS + <i>Staph. aureus</i>
52	4x CNS	Type II	CNS
8	CNS <i>Staph. aureus</i>	Type IV	

also consider the four patients, with a PM indicative of infection, where a bacterial isolation was only possible through SFC. However, the authors emphasise the need for a critical interpretation of all available clinical data to confirm or dismiss the diagnosis of PJI.

A further point for discussion is the isolation of multiple bacterial species from various patients. In seven of 26 patients with positive SFC, Tunney et al. isolated multiple bacterial species [12]. Achermann et al. isolated polymicrobial infections in six of 37 patients [1]. Trampuz et al. reported multiple bacterial species in 11 of 79 cases [11]. In our study we isolated multiple bacterial species in eight of 59 patients. Out of these eight cases five were defined as PJI, and in three the isolation of multiple pathogens could also be confirmed by intraoperative tissue samples (Table 4).

The main advantage of SFC is the identification of the responsible pathogen. As with all microbiological methods there is a risk of contamination when sonication is performed in an inadequate manner. For example, Trampuz et al. reported an increased risk of contamination when performing sonication using plastic bags [10]. Nevertheless, the authors stress the need for a critical interpretation of all microbiological results obtained.

Both the reduced metabolic state of the sampled bacteria which imparts the sonication samples with the necessary robustness to withstand extended transportation times and the simple handling of the sonication process facilitate the integration of sonication into the existing diagnostic routine of a clinic. The culture costs of sonication samples are also comparable to the cost of conventional intraoperative samples. Since there are often fewer sonication samples than acquired intraoperative tissue cultures, the cost for sonication is less than that for the conventional microbiological methods. The authors are convinced that the additional diagnostic information afforded through SFC clearly outweighs the additional cost and justify the clinical feasibility of sonication.

In conclusion in our patient group SFC was the microbiological method with the highest sensitivity. SFC was the diagnostic method which was able to identify the most cases of PJI and was superior to the PM. The major drawback of sonication is the risk of contamination and the resulting uncertainty in the interpretation of positive bacterial isolates in a single sample. To solve this diagnostic dilemma we recommend the combined sampling of both PM and SFC. Only when the histological and the microbiological results are interpreted in combination with the clinical presentation can a qualified diagnosis regarding PJI be made.

We recommend the inclusion of SFC in the diagnostic routine for the diagnosis of PJI.

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Conflict of interest The authors declare that they have no conflict of interest.

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