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INFECTION

Effects of different tissue specimen pretreatment methods on microbial culture results in the diagnosis of periprosthetic joint infection

X. Fang,
L. Zhang,
Y. Cai,
Z. Huang,
W. Li,
C. Zhang,
B. Yang,
J. Lin,
P. Wahl,
W. Zhang

From The First Affiliated Hospital of Fujian Medical University, Fuzhou, China

Aims

Microbiological culture is a key element in the diagnosis of periprosthetic joint infection (PJI). However, cultures of periprosthetic tissue do not have optimal sensitivity. One of the main reasons for this is that microorganisms are not released from the tissues, either due to biofilm formation or intracellular persistence. This study aimed to optimize tissue pretreatment methods in order to improve detection of microorganisms.

Methods

From December 2017 to September 2019, patients undergoing revision arthroplasty in a single centre due to PJI and aseptic failure (AF) were included, with demographic data and laboratory test results recorded prospectively. Periprosthetic tissue samples were collected intraoperatively and assigned to tissue-mechanical homogenization (T-MH), tissue-manual milling (T-MM), tissue-dithiothreitol (T-DTT) treatment, tissue-sonication (T-S), and tissue-direct culture (T-D). The yield of the microbial cultures was then analyzed.

Results

A total of 46 patients were enrolled, including 28 patients in the PJI group and 18 patients in the AF group. In the PJI group, 23 cases had positive culture results via T-MH, 22 cases via T-DTT, 20 cases via T-S, 15 cases via T-MM, and 13 cases via T-D. Three cases under ongoing antibiotic treatment remained culture-negative. Five tissue samples provided the optimal yield. Any ongoing antibiotic treatment had a relevant influence on culture sensitivity, except for T-DTT.

Conclusion

T-MH had the highest sensitivity. Combining T-MH with T-DTT, which requires no special equipment, may effectively improve bacterial detection in PJI. A total of five periprosthetic tissue biopsies should be sampled in revision arthroplasty for optimal detection of PJI.

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Keywords: Pretreatment, Culture, Periprosthetic joint infection

Article focus

- This manuscript focuses on different pretreatment methods of periprosthetic tissue samples to improve diagnosis of PJI.

Key messages

- Five different pretreatment methods for the microbiological workup of tissue biopsies were compared regarding performance in the diagnosis of PJI. The present study demonstrated that tissue-mechanical homogenization had the highest sensitivity. Pretreatment with dithiothreitol appeared to be impervious to ongoing antibiotic treatment, with good sensitivity throughout. Sonication of tissue samples appeared to have a high sensitivity in the absence of antibiotic treatment.
- For optimal diagnostic performance, at least four and no more than five biopsies of periprosthetic tissues should be sampled for microbiological cultures. This study

Correspondence should be sent to Wenming Zhang; email: zhangwm0591@fjmu.edu.cn

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confirms recent publications regarding the optimal number of samples.
- In case of tissue sampling under ongoing antibiotic treatment, pretreatment of the sample with dithiothreitol might increase the sensitivity of microbiological cultures. This might be combined with mechanical homogenization, potentially increasing further the sensitivity.

Strengths and limitations

- This is the first study comparing sensitivity and specificity of various pretreatment methods in the microbiological workup of periprosthetic tissue biopsies to diagnose PJI.
- This study involved a single centre, with a relatively small sample size, and a relatively large proportion of patients sampled under antibiotic treatment. Sensitivity and specificity might be different in another setting.
- A subgroup analysis of the patients without ongoing antibiotic therapy was performed to account for this important confounding factor.
- Combinations of pretreatments would be possible, such as the combination of pretreatment with dithiothreitol and mechanical homogenization, and may possibly improve sensitivity. However, this was not tested explicitly in our study.

Introduction

Periprosthetic joint infection (PJI) is a serious complication after arthroplasty that imposes a heavy toll on the affected patients as well as on the healthcare system, consequently placing a considerable economic burden on society.[1-5] Serum inflammatory biomarkers have low discrimination and limited usefulness in PJI diagnosis.[6,7] Molecular diagnostic techniques and synovial fluid biomarkers may perform well to identify PJI, but provide only limited identification of the causative microorganism and possible resistances to antibiotics.[8,9] Thus, microbiological culture of periprosthetic samples or of retrieved components remains the mainstay in PJI diagnosis. Both the Musculoskeletal Infection Society (MSIS) as well as the Infectious Diseases Society of America (IDSA) consider microbiological cultures as the primary indicator of PJI, but acknowledge the limitations of the method and consider other diagnostic criteria.[10-12]

Many factors in the collection and processing of specimens are known to influence culture results.[13-17] Specimens for microbiological culture can be obtained from various sources, such as native joint fluid (N-JF), sonication fluid (SF) of retrieved implants, and periprosthetic tissues.[18] However, sufficient N-JF may not always be obtained,[19,20] and sonication is technically demanding and not always available.[21] In contrast, obtaining tissue samples intraoperatively is relatively easy and thus becomes the main source of samples for microbiological workup. However, the sensitivity of tissue cultures has been reported previously to range approximately from 40% to 80%.[15,22] Therefore, it is of great significance to study how optimization of pretreatment methods of tissue specimens may improve detection of microorgansisms.[15,22,23]

One of the reasons for reduced sensitivity might be that bacteria persist intracellularly[24,25] or in biofilm,[16,21] requiring pretreatment of the sample to release the microorganisms. Direct culture or manual milling is routinely used to pretreat tissue samples in clinical practice.[26] Homogenizing tissue samples before culture has been shown to increase sensitivity and simultaneously reduce incidence of contamination.[27] It is well acknowledged that the existence of biofilm is an important factor for low sensitivity of cultures, and several strategies have been developed to disrupt it and reactivate the included microorganisms.[16,21] DL-dithiothreitol (DTT), which inhibits the biosynthesis of intercellular polysaccharides, can dissolve biofilms formed on surfaces, increasing the release of bacteria.[28,29] Furthermore, the application of sonication has been proven to effectively disrupt biofilms, but there have been no reports on its application to biofilms formed within tissues and the consequent effect on sensitivity of microbiological culture. Overall, comparative evaluation of tissue pretreatment methods is lacking so far. Accordingly, we attempted to evaluate different tissue pretreatment methods and develop an optimal pretreatment protocol.

Methods

This study was performed in a single tertiary reference university centre. We included patients > 18 years of age who underwent total hip arthroplasty (THA) or total knee arthroplasty (TKA) revision between December 2017 and September 2019. This study was a prospective continuous series. Exclusion criteria were: 1) insufficient samples obtained intraoperatively, with fewer than six periprosthetic tissue samples and no N-JF or SF; 2) samples that were contaminated or suspected of contamination during sample collection or processing; and 3) metal-on-metal pairing, periprosthetic fracture, or microcrystalline arthritis, which might cause purulence and acute inflammation, leading to misdiagnosis of PJI. A PJI was defined following the clinical decision, as well as reclassified retrospectively according to both the MSIS and IDSA criteria (Supplementary material).[11,12]

Aspirated N-JF was obtained intraoperatively, before capsulotomy. All specimens were transported to the laboratory within 30 minutes. White blood cell count (N-JF-WBC) and polymorphonuclear cell (N-JF-PMN) count were carried out on 0.5 ml of N-JF. A 1 ml aliquot of N-JF was added to a Bactec Plus/F aerobic culture bottle (9239513; Becton-Dickinson, Franklin Lakes, New Jersey, USA) and a Bactec Lytic/10/F anaerobic culture bottle (9293496; Becton-Dickinson) and cultivated in a Bactec automatic incubator (FX400; Becton-Dickinson) for 14 days.

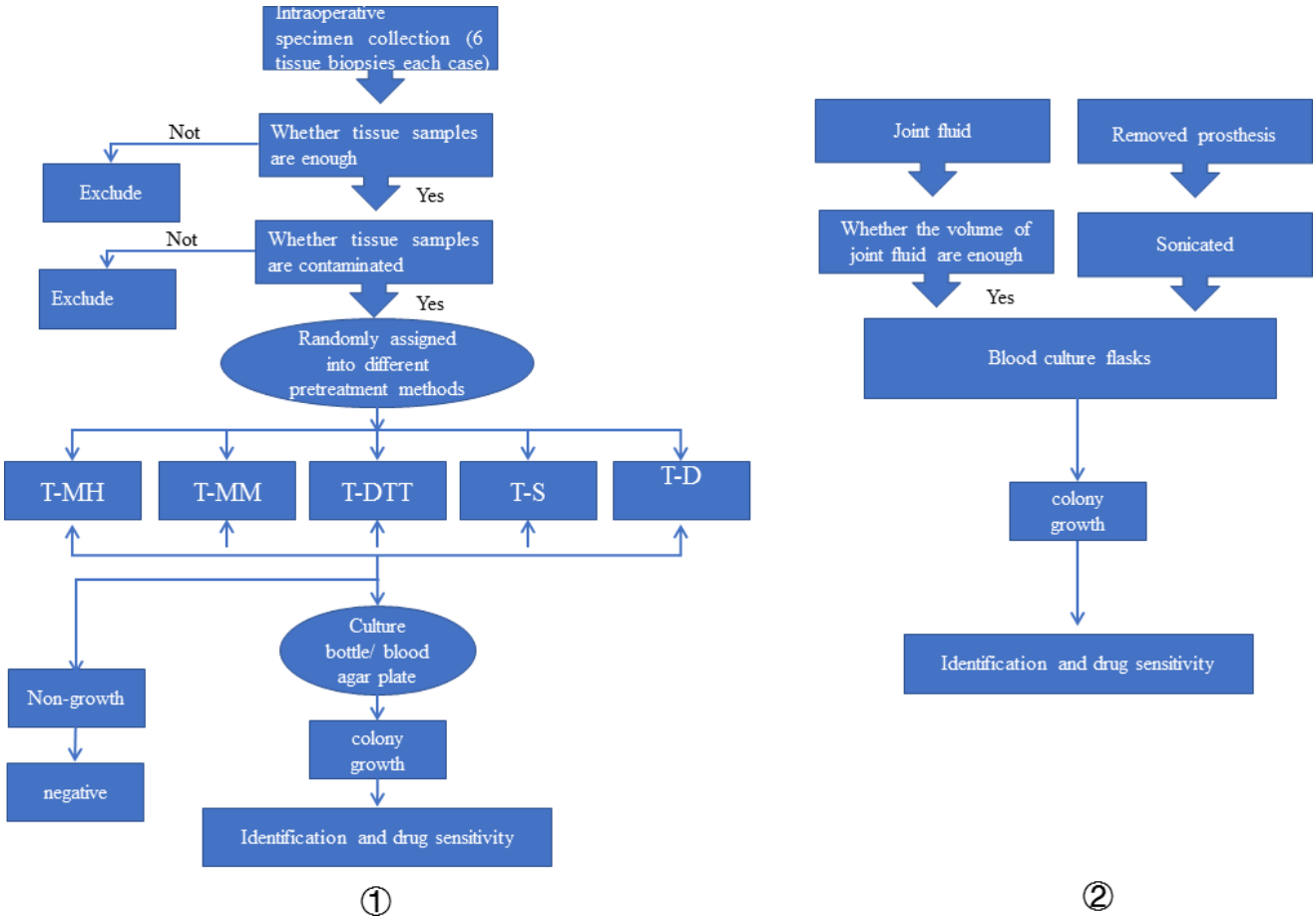

Fig. 1

Workflow of the microbiological sample workup. Only cases with complete sampling, including six biopsies of periprosthetic tissues, and collection of sufficient synovial fluid and of prosthetic components for sonication were included. The tissue biopsies were subdivided into six fragments, randomly assigned to the five pretreatment methods for microbiological workup (tissue-mechanical homogenization (T-MH), tissue-manual milling (T-MM), tissue-DL-dithlothreltol (T-DTT), tissue-sonication (T-S), and tissue-direct (T-D), as described) as well as for histology.

The retrieved prosthetic components were placed in a sterilized plastic box with approximately 400 ml sterile saline, sonicated at 40 Hzfor five minutes (VS-TP24 Ultrasonic cleaner; Jiangsu Wuxi Woxin Instruments, Shanghai, China). The fluid was then centrifuged at 4,000 rpm for five minutes. The supernatant was discarded, and the concentrate was resuspended in sterile phosphate-buffered saline (PBS). One millilitre of resuspension was added to a Bactec Plus/F aerobic culture bottle and a Bactec Lytic/10/F anaerobic culture bottle and incubated for 14 days as mentioned above.

Six periprosthetic tissues biopsies were collected intraoperatively. These were subdivided randomly into samples for routine pathological examination as well as for the five different tissue pretreatment methods (T-MH, T-MM, T-DTT, T-S, and T-D) for microbiological workup. Samples were transported in dry tubes, and workup performed within 30 minutes, starting with pretreatment with pre-chilled culture medium. For T-MH, tissue samples were placed in sterile centrifuge tubes with 5 mL brain heart broth, vortexed and shaken for 15 minutes, and homogenized for 60 to 90 seconds in a fully automated rapid grinding instrument (JXFSTPRP-24; Jingxin Industrial, Shanghai, China) set at 40 Hz. For T-MM, tissue samples were placed in centrifuge tubes with 5 ml brain heart broth and manually ground with a disposable sterile grinding rod until macroscopic homogenization. For T-DTT, tissue samples were immersed in centrifuge tubes with 5 mL DTT following a previously published protocol,[30] and vortexed for 15 minutes, after which 5 ml brain heart broth was added. For T-S, samples were placed in sterile centrifuge tubes with 5 ml brain heart broth, shaken for 30 seconds, sonicated at 40 Hz for five minutes (VS-TP24 Ultrasonic cleaner), and vortexed for 30 seconds. Microbiological aerobic and anaerobic cultures were performed on blood agar plates. For T-D, tissue samples were incubated directly on blood agar plates.

The workflow of the microbiological sample workup is illustrated in Figure 1. The Vitek II system (Biomerieux, Durham, North Carolina, USA) was used for species identification and drug sensitivity testing.

Table I. Summary of demographic data and clinical features of the patients included.

Characteristics	PJI group (n = 28)	AF group (n = 18)	p-value
Mean age, yrs (SD)	65.2(8.90)	62.9(13.7)	0.757*
Sex, male:female, n (%)	13:15 (36:64)	7:11 (39:61)	0.402†
Median follow-up time, mths (IQR)	26.6 (14 to 42)	28.7 (8 to 40)	0.861‡
Joints, hips:knees, n (%)	15:13 (54:46)	13:5 (72:28)	0.684†
Sinus tract, n (%)	18 (64)	0 (0)	< 0.001†
Ongoing antibiotic treatment, n (%)	13 (46)	0 (0)	0.003†
Median CRP, mg/l (IQR)	49 (2 to 96)	5 (2 to 11)	< 0.001‡
Median ESR, mm/h (IQR)	77 (15 to 130)	38.5 (12 to 72)	< 0.001‡
Median volume of N-JF, ml (IQR)	4 (1 to 8)	5 (2 to 9)	0.367‡
Median N-JF-WBC, × 10^6/l (IQR)	4,369 (869 to 43,148)	711 (124 to 2,754)	< 0.001‡
Median N-JF-PMN, % (IQR)	86 (54 to 93)	51 (5 to 69)	< 0.001‡

*Independent-samples *t*-test.
†Chi-squared test.
‡Mann–Whitney U test.
IQR, interquartile range; N-JF, native joint fluid; PMN, percentage of polymorphonuclear granulocytes; WBC, white blood cell count.

Statistical analysis. Data were analyzed using SPSS 21.0 (IBM, Armonk, New York, USA). Results were expressed as the mean ± SD for normally distributed data and the median (interquartile ranges (IQRs)) for non-normally distributed data. Independent-samples *t*-test, Mann-Whitney U test, chi-squared test, or Fisher's exact test were used to compare differences, as appropriate considering data characteristics. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the various pretreatment methods were calculated. A p-value < 0.05 was considered statistically significant. Culture results of single samples such as N-JF and SF were considered binary as positive or negative. For N-JF-WBC, the cut-off was set at 2,000 × 10^6 leucocytes/l. For N-JF-PMN, above 65% was considered as compatible with PJI. For histology, a cut-off of 5 PMNs/HPF was accepted. All possible combinations of randomly chosen biopsies were considered to calculate sensitivity and specificity for less than the maximum number of biopsies, but with a minimum of two samples. Graphical illustration of results was performed with R Graphics Package Version 3.6.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The gold standard for diagnosis of PJI for this analysis was the clinical decision. Sensitivity and specificity analysis was done for the whole cohort of patients, and separately for those patients without any ongoing antibiotic treatment as well as for those with ongoing antibiotic treatment.

Results

In all, 55 patients undergoing revision arthroplasty for PJI or AF between December 2017 and September 2019 in our centre were screened. Four cases were excluded because of insufficient N-JF samples, four due to no SF samples, and one due to sample contamination. Thus, 46 cases could be included: 28 patients (61%) with PJI and 18 patients (39%) with AF. The demographic characteristics of the patients are presented in Table I. The proportions of patients who received antibiotic treatment and presented with a sinus tract were higher in the PJI group than those in the AF group. ESR, CRP, N-JF-WBC, and N-JF-PMN% were also more elevated. Details of diagnostic criteria and classification following the clinical decision, as well as a retrospective classification following the IDSA and the MSIS criteria, are provided in the Supplementary Material. Discrepancies between the different diagnostic criteria happened only in isolated cases, as illustrated in Figure 2. Considering the details of the individual results and the small differences in defining the gold standard between the different classifications, only the clinical decision was considered for further analysis of sensitivity and specificity.

The culture results are provided in Table II. Pathogens were isolated from 15 (54%), 18 (64%), 23 (82%), 22 (79%), 15 (54%), 20 (71%), and 13 cases (46%) in the N-JF, SF, T-MH, T-DTT, T-MM, T-S, and T-D groups, respectively. There were six cases with false-positive culture results in the AF group, three cases in the T-MM group, two cases in the T-S group, and one case each in the N-JF, T-MH, and SF groups; there were no cases in the T-DTT group. Details are provided inSupplementary Material.

The diagnostic efficiencies of different single diagnostic criteria, such as N-JF, SF, N-JF-WBC, N-JF-PMN, and histology are listed in Table III. As the identification of phenotypical microorganisms in at least two samples is a requirement of the usual guidelines for diagnosis, sensitivity and specificity of tissue biopsies were performed for at least two samples and for a random selection of samples up to the total of six samples. Due to the minimum of two samples, there were no false-positive results. Thus, analysis was limited to sensitivity, as specificity was always 100%. Results illustrating the influence of the number of samples and of ongoing antibiotics are provided in Figure 3. Both the number of samples analyzed and any ongoing antibiotic treatment had a great influence on the sensitivity

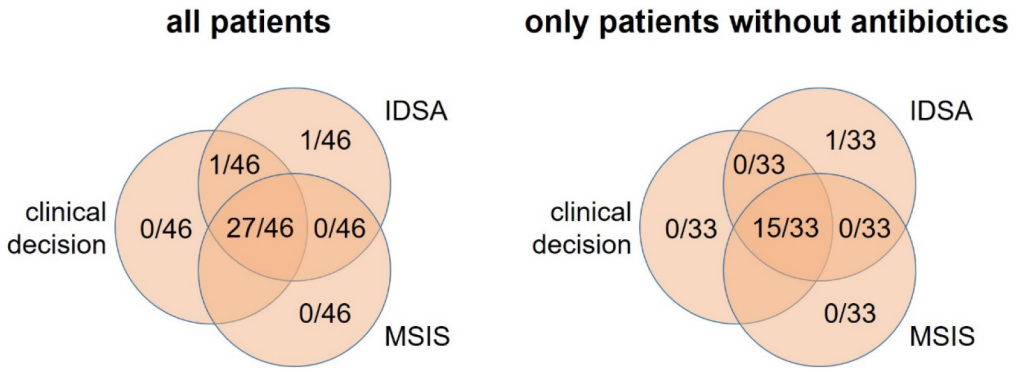

Fig. 2

Distribution of patients included following the clinical decision, and retrospective reclassification following the Infectious Disease Society of America (IDSA) as well as the Musculoskeletal Infection Society (MSIS) criteria for diagnosis of periprosthetic joint infection (PJI). Left: all cases included in the study. Right: only patients without ongoing antibiotic treatment. Note that the MSIS criteria missed one infection in a patient under antibiotic treatment for PJI. The IDSA overdiagnosed one case classified clinically as aseptic failure, due to a positive sonication result. The clinical follow-up however permits confirmation of aseptic failure, as no infection was observed during follow-up.

Table II. Summary of the causative microorganisms identified in the cases with periprosthetic joint infection. The clinical decision was used as the gold standard for identification of cases. No polymicrobial infections were identified. Details of which culture results yielded identification of the causative microorganisms are provided in Supplementary Table i.

Microorganism	n (%)
Staphylococcus aureus	5 (18)
Staphylococcus epidermidis	3 (11)
Staphylococcus lugdunensis	1 (4)
Streptococcus agalactiae	1 (4)
Enterococcus faecalis	1 (4)
Enterococcus gallinarum	1 (4)
Finegoldia magna	1 (4)
Helcococcus kunzii	1 (4)
Aggregatibacter aphrophilus	1 (4)
Klebsiella pneumoniae	1 (4)
Escherichia coli	1 (4)
Pseudomonas aeruginosa	1 (4)
Mycobacterium abscessus	2 (7)
Mycobacterium fortuitum	1 (4)
Culture-negative PJI	3 (18)
Total number	15 (100)

PJI, periprosthetic joint infection.

of microbiological tissue sample cultures. The sensitivity of T-MH was highest compared to any other pretreatment method. In the absence of any antibiotic treatment, T-S performed just as well. The sensitivity of T-DTT appeared to be impervious to any ongoing antibiotic treatment but performed less well than T-MH in the absence of antibiotics. T-MM performed much worse than T-MH, close to the sensitivity of T-D, the method with the lowest sensitivity throughout.

Discussion

Diagnosis of PJI is based mainly on microbiological culture results.[11,12] This not only proves the presence of microorganisms, but also remains the only method to reliably identify antibiotic resistance.[6-9] Due to the limitations of culture methods, many challenges remain in the diagnosis of PJI.[23] In clinical practice, as sufficient N-JF cannot always be obtained,[19,20] and as SF is not widely available,[16,21,31] periprosthetic tissue biopsies remain the main source of microbiological samples.[15,18,22,32] However, the sensitivity of microbiological tissue culture may be reduced due to several reasons, among them a failure to collect sufficient tissue samples,[23,32] biofilm formation within living tissues,[17,33-35] the presence of fastidious bacteria and intracellular persistence within tissues,[24,25] as well as the administration of antibiotics before sampling.[21] Different pretreatment methods of tissue biopsies were compared, in order to determine optimal microbiological culture performance to diagnose PJI.

The present study demonstrated that different pretreatment methods could significantly improve the yield of microbiological cultures of periprosthetic tissue biopsies (Figure 3). The sensitivity of T-MH was higher than that of all other pretreatment methods. Any ongoing antibiotic treatment however had a detrimental effect on sensitivity, except for T-DTT. Technically, T-DTT can easily be combined with T-MH, potentially increasing sensitivity even further. However, this remains hypothetical, as it was not tested explicitly. T-S also appeared to have a high sensitivity, at least when sampling was performed in the absence of any ongoing antibiotic treatment. T-MM and T-MH were used to disrupt tissues and cells, in order to release bacteria present in the tissues in close proximity to the prosthesis, which are mostly intracellular persisters.[24,25] T-MH appeared to have a far better sensitivity as T-MM (Figure 3), while being technically easier and less sensitive to contamination, as it requires considerably fewer manipulations.[27] T-DTT and T-S were applied to disrupt biofilms, as biofilm formation does not necessarily require an inert surface and may form within living tissues.[33-36] While T-S appeared to have a very good sensitivity on samples taken in the absence of any ongoing antibiotic treatment, T-DTT appeared not to be affected

Table III. Summary of the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of N-JF (native joint fluid culture), sonication fluid culture, normal synovial fluid white blood count, percentage of polymorphonuclear neutrophil granulocytes among normal synovial fluid, and histology against the gold standard of the clinical decision. Results are provided separately for all patients included, as well as for the subgroups of patients without antibiotic treatment and with antibiotic treatment. For the N-JF-WBC, the cutoff was at 2,000 leucocytes/µl. For N-JF-PMN, it was > 65%. For histology, it was > 5 PMN/high-powered field.

All patients					
Parameter	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
N-JF	53.6	94.4	93.8	56.7	69.6
SF	64.3	94.4	94.7	63.0	76.1
N-JF-WBC	100.0	94.4	96.6	100.0	97.8
N-JF-PMN	85.7	83.3	88.9	78.9	84.8
Histology	96.4	100.0	100.0	94.7	97.8
Patients without antibiotic treatment					
N-JF	60.0	94.4	90.0	73.9	78.8
SF	80.0	94.4	92.3	85.0	87.9
N-JF-WBC	100.0	94.4	93.8	100.0	97.0
N-JF-PMN	86.7	83.3	81.3	88.2	84.8
Histology	100.0	100.0	100.0	100.0	100.0
Cases with antibiotic treatment*					
N-JF	46.2	N/A	100.0	0.0	46.2
SF	46.2	N/A	100.0	0.0	46.2
N-JF-WBC	100.0	N/A	100.0	N/A	100.0
N-JF-PMN	84.6	N/A	100.0	0.0	84.6
Histology	92.3	N/A	100.0	0.0	92.3

*In the subgroup of patients with ongoing antibiotic treatment, specificity cannot be calculated, as no cases of aseptic failure were included.
N-JF, native joint fluid; NPV, negative predictive value; PMN, percentage of polymorphonuclear neutrohpil granulocytes; PPV, positive predictive value; SF, sonication fluid; WBC, white blood cell.

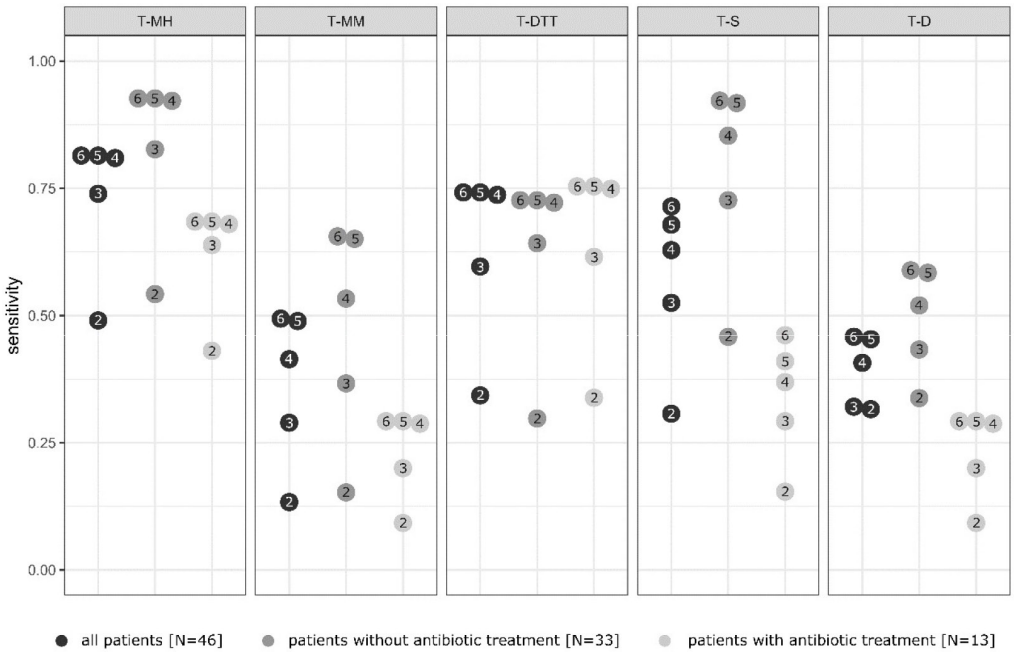

Fig. 3

Illustration of the sensitivity of the microbiological culture results of periprosthetic tissue biopsies for diagnosis of periprosthetic joint infection (PJI), separated by pretreatment method. For this analysis, PJI was accepted with at least two samples positive for phenotypically identical microorganisms, against the gold standard of the clinical decision. As the criteria of two positive samples eliminated all false-positive culture results, specificity was always 100%. Thus, only sensitivity is illustrated, separately for all patients included, as well as for the subgroups without or with ongoing antibiotic treatment. The numbers indicate how many samples were considered, among the maximum of six biopsies sampled. For numbers smaller than the maximum of six, all possible iterations were considered. T-D, direct culture of the tissue sample without pretreatment; T-DTT, tissue pretreatment with dithiotreitol; T-MH, tissue mechanical homogenization; T-MM, tissue mechanical milling; T-S, tissue sonication.

by antibiotics (Figure 3). The good performance of T-S may be seen as surprising, as sonication disrupts biofilm by cavitation, which requires interruption of the ultrasonic waves by a hard interface, an effect not observed on soft materials.[37] Both T-MH and T-S offer a supplementary advantage. The fluid obtained may be inoculated in blood culture flasks, which offer a better sensitivity and a faster recovery than cultures on agar plates.[14,22,32,38,39] The yield of T-D was low, as expected, considering low surface-to-volume ratio of the sample and reduced contact with the culture medium.

As the accuracy of each individual microbiological sample is not perfect, the number of samples analyzed has an influence on the global sensitivity and specificity of the diagnosis of PJI.[15,22,32,40,41] In this study, at least four samples for microbiological workup are required for optimal sensitivity (Figure 3). Increasing this number to six did not increase sensitivity further. The number of five biopsies is in line with other publications,[32,42] but the reduced need, observed by another group, of only three samples when using blood culture flasks could not be confirmed.[40] The results from this study confirm the classical guidelines recommending four to five samples.[12] This number is not necessarily given solely by culture technique, but may also be explained by non-random distribution of bacteria within tissues, something particularly relevant to low-virulence microorganisms.[43] Biopsies should be sampled from peri-implant tissues, or better inflammatory membranes formed around loose or infected implants.[18]

Prechilled culture medium was used to reduce the probability of bacterial inactivation.[17] In addition, blood culture bottles were used in this study due to the higher sensitivity and shorter cultivation time.[13,14,22,32,39] Cultures were maintained for 14 days, to recover slow-growing microorganisms.[44] However, blood culture flasks usually allow the recovery of only one strain, the first one to overgrow the fluid medium. In this study, no polymicrobial infections were identified, whereas this would be expected in up to 15% of PJI cases.[15,20,32] Nevertheless, the sole use of blood culture flasks is also reported as performing better than agar plate cultures.[40] Sonication was limited to five minutes with appropriate energy and frequency settings, to avoid inactivation of microorganisms.[45] However, the performance of sonication was rather poor compared to other studies, with a sensitivity of 64% and a specificity of 94% (Table III).[16,21,22] Sonication may not be the optimum modality, as bacteria are mainly intracellular in the tissues in close proximity to the implant.[18,24,25]

This study only focused on microbial culture results of various pretreatment methods, but there were some culture-negative PJI cases. Accordingly, molecular diagnostics, such as polymerase chain reaction (PCR) and metagenomic next-generation sequencing (mNGS), should be taken into consideration to improve PJI diagnosis.[46,47] Even if microbiological culture techniques could be optimized,[32,40,48] there were very few cases with discrepant diagnoses (Figure 2), and the chosen gold standard of the clinical decision always was confirmed by follow-up. For this reason, the analysis of sensitivity and specificity was done using the clinical decision as the gold standard. There are well-known issues with both the IDSA and the MSIS criteria; the MSIS criteria in particular miss some low-grade infections.[10,23,49] In this series, the MSIS criteria misdiagnosed one obvious case of PJI due to ongoing antibiotic treatment at sampling. Potential issues with culture technique thus should not have had a notable influence on the conclusions regarding sensitivity and specificity. As previously shown, the preoperative administration of antibiotics has a major impact on the sensitivity of microbiological cultures.[21,23]

Although pretreatment methods can improve bacterial detection, contamination issues should also be considered. Although there were no statistically significant differences in specificity among various pretreatment methods, as a minimum of two positive samples was a diagnostic requirement, T-MM and T-S had a higher incidence of false-positive cultures. These were always isolated and could thus be easily identified as such (Supplementary Table iSupplementary material). The process of T-MH, including the manual addition of broth and the use of a grinding instrument, is a tedious and time-consuming process, during which microorganisms in the broth, air, and grinding instrument might be introduced, and hence contamination may occur.[27] Conversely, the process of T-DTT is relatively simple, and the specificity was 100%.[50] The combination of T-MH and T-DTT may be useful to improve sensitivity, while not altering specificity. This however remains hypothetical, as it was not tested explicitly.

The sensitivity of the N-JF microbial culture in this study was only 53.6%, possibly because insufficient N-JF samples were obtained due to the 'dry tap'; as shown previously,[19,20] clump formation of bacteria in N-JF might partly explain the poor results.[18,51] N-JF-WBC and N-JF-PMN however performed much better in identifying PJI, as did the quantification of PMN by histology, in line with previously published studies.[11,12,15,16,19,20,23,52-55] This strengthens the accuracy of our study. While any ongoing antibiotic treatment causes a major drop in culture sensitivity, N-JF-WBC, N-JF-PMN, and histology retained their diagnostic accuracy (Table III).

The fact that this study was performed in a single centre, with a rather small number of patients, must also be considered. Sensitivity and specificity of diagnostic tests are determined by the patient population studied. In this case series, there was a high prevalence of patients with established sinus tract and a high proportion of patients under ongoing antibiotic treatment when sampling was performed (Table I). The proportion of highly virulent microorganisms was also slightly higher than usual in PJI. Thus, sensitivity and specificity may vary in other settings.

Supplementary material

Details for all cases and periprosthetic joint infection diagnostic criteria.

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Author information:
- X. Fang, MD, PhD, Orthopaedic Surgeon
- L. Zhang, MD, Orthopaedic Surgeon
- Y. Cai, MD, Orthopaedic Surgeon
- Z. Huang, MD, PhD, Orthopaedic Surgeon
- W. Li, MD, Orthopaedic Surgeon
- C. Zhang, MD, PhD, Orthopaedic Surgeon
- J. Lin, MD, PhD, Orthopaedic Surgeon
- W. Zhang, MD, PhD, Orthopaedic Surgeon

Department of Orthopaedic Surgery, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China.
- B. Yang, MD, Chief Technologist, Department of Laboratory Medicine, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China.
- P. Wahl, MD, PhD, Orthopaedic Surgeon, Division of Orthopaedics and Traumatology, Cantonal Hospital Winterthur, Winterthur, Switzerland.

Author contributions:
- X. Fang: Wrote the manuscript, Performed the study
- L. Zhang: Performed this study.
- Y. Cai: Performed the study, Conducted the statistical analysis.
- Z. Huang: Edited the manuscript.
- W. Li: Conducted the statistical analysis.
- C. Zhang: Translated the manuscript into English.
- B. Yang: Guided the microbial culture methods.
- J. Lin: Edited the manuscript.
- P. Wahl: Edited the manuscript, Conducted the statistical analysis.
- W. Zhang: Designed the study.

- X. Fang, L. Zhang and Y. Cai contributed equally to this work.

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