Peer Review File

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Reviewer A

1、Comment: I think it is very important to rule out other causes of aseptic loosening (such as possible allergic reactions, poor component positioning, mechanical wear), before performing a revision arthroplasty or spinal fusion procedure.

Response: Thank you for your valuable suggestion.

This article was designed to estimate the yield of sonication fluid culture for detecting the presence of microorganisms in orthopedic devices with a presumed diagnosis of aseptic loosening and to summarize the clinical characteristics and outcomes of these patients with unexpected positive results. Therefore, eligible studies were peer-reviewed publications containing empiric data on the yield of sonication fluid culture for human patients whose orthopedic devices (artificial joints, osteo-synthetic materials, pedicle screws) were removed for loosening without evidence of infection such as sinus tract, highly elevated serum biomarkers, local swelling and fever.

All patients included in this study had presumed aseptic loosening. We agree that it is extremely important to rule out other causes of aseptic loosening (such as possible allergic reactions, poor component positioning, or mechanical wear) before performing a revision arthroplasty or spinal fusion procedure, and we have emphasized this point in the revised manuscript.

2、**Comment:** In my opinion, sonication is a useful tool in the diagnosis of low virulent infections with low suspicion or in patients with high suspicion where the causative germ could not be found. However, the study of aseptic loosening may have uncertain results, and I believe that the diagnosis of periprosthetic infection is currently determined by a positive culture, to my knowledge.

Response: Thank for your valuable suggestion.

As previously mentioned, low virulent microorganisms, especially coagulase-negative staphylococcus, account for the majority of identified bacteria in sonication fluid culture for presumed aseptic loosening of both artificial joints and spinal instruments. Sonication culture of explanted endoprostheses, which is considered to be a more sensitive method than traditional tissue culture, may serve as a useful tool in the diagnosis of low-suspicion patients with low virulent infections or in highly suspected patients for whom the causative microorganism cannot be determined.

Meanwhile, it is true that the study of aseptic loosening may produce uncertain results and the diagnosis of periprosthetic infection is currently determined by a positive culture. In this study, we mainly focused on the yield of sonication fluid culture in cases of presumed aseptic loosening of orthopedic devices.

We agree with your opinion and have emphasized these points in the revised manuscript.

3、Comment: In your study, I find a series of determining limitations, related mainly

to these factors. I find too high heterogeneity of studies, possibly due to too broad inclusion criteria. I would recommend that you differentiate the different methods that the authors have used to define aseptic loosening, as well as the material in question, since vertebral screw loosening does not have the same rates or the same causes as a total knee or hip arthroplasty, for example.

Response: T Thank you for your valuable suggestion.

We observed that high heterogeneity existed in included studies during data pooling and attempted to explore the source of heterogeneity. Therefore, we conducted subgroup analyses according to the type of orthopedic device and the duration of sonication and vortexing.

In 4 (1-4) studies, the total duration of sonication and vortexing was ≤5 minutes, and the pooled yield was 49% (95% CI: 41–56%). No significant heterogeneity (I^2 = 31%) was observed across these studies. For studies(2, 5, 6) with a total sonication and vortexing duration of >5 minutes and ≤10 minutes, the pooled yield was 21% (95% CI: 13–29%; I^2 = 31%). Nguyen et al. sonicated explanted endoprostheses for 30 minutes, with which the yield of sonication fluid culture was only 5% (7). In these subgroup analyses, no significant heterogeneity was identified. I^2=31% (<50%) indicated insignificant heterogeneity, which was acceptable for a meta-analysis.

Subgroup analysis revealed that the significant heterogeneity seen across the included studies mainly resulted from differences in the duration of sonication and vortexing. A total duration of more than 5 minutes will significantly decrease the yield of sonication fluid culture. This may be related to the reduced viability or even destruction of detached bacteria. The use of an ultrasound bath for 1–5 minutes at 40

kHz may yield the optimal diagnostic performance for bacterial colonization (49%, 95% CI 41–56%). This indicated that the significant heterogeneity across included studies mainly came from the difference in duration of sonication and vortexing. Meanwhile, with the extension of the duration of sonication and vortexing, the positive rate of sonication fluid culture gradually decreased.

Meanwhile, subgroup analysis was conducted according to the type of orthopedic devices with presumed aseptic loosening. Two(1, 2) studies involving a total of 114 patients reported the yield of sonication fluid culture for detecting microorganisms from spinal fusion instrumentations. No significant heterogeneity ($I^2 = 0\%$) was found between these studies, and the pooled yield was 42% (95% CI: 33–51%). A further 6(3-8) studies involving a total of 307 patients reported the yield of sonication fluid culture in artificial joints. Significant heterogeneity ($I^2 = 91\%$) was observed across these studies, and the pooled yield for detecting the presence of microorganisms was 29% (95% CI: 13–47%)

We agree with your opinion and have emphasized these points in the revised manuscript.

4、Comment: I think studies where it is not clear what the definition of aseptic loosening is should have been excluded.

Response: Thank you for your valuable suggestion.

All included studies provided methods to define aseptic loosening. In Table 1 of the original manuscript, a row was named “definition of loosening”, which was inaccurate. In fact, we aimed to state that three studies (2, 6, 8) did not detail the

imaging method (CT or X-ray film) used to detect the loosening of orthopedic devices. We have deleted the relevant statements in the revised manuscript. Meanwhile, Table 1 has been revised, with this row renamed as “methods to detect loosening”, and the relevant data have been extracted from the original studies.

We apologize for this mistake and believe that the correction improves the quality of this manuscript.

5、Comment: I think that the PCR diagnosis of sonication has a different sensitivity and specificity, and on the other hand, I would recommend excluding it from the study, or in any case studying it separately.

Response: Thank you for your valuable suggestion.

In the quantitative analysis section, we only included studies that used sonication fluid culture of explanted orthopedic devices. The PCR diagnosis of sonication fluid was discussed in the section of qualitative analysis. Meanwhile, we have discussed the differences between these two methods in the revised manuscript.

Bereza et al. published two (5, 9) studies with overlapping patients but adopted separate microbiological tests (sonication fluid culture and sonication fluid PCR); therefore, one study (9) was analyzed quantitatively and the other (5) qualitatively.

6、Comment: I do not agree with the phrase online 287-290, because I think that more positive results in the sonication of aseptic loosening materials does not always imply periprosthetic infection, since it can be colonization.

Response: Thank you for your valuable suggestion.

In this study, we aimed to detect the presence of microorganisms in presumed aseptic loosening of orthopedic devices. More positive results in the sonication fluid culture of aseptic loosening materials does not imply periprosthetic joint infection. In fact, it only indicates bacterial colonization or subclinical infection. This point has been emphasized in the revised manuscript.

7、Comment: I do not agree with the sentence of line 313-314, since your results show heterogeneity also in practically all the subgroups of analyzes carried out.

Response: Thank you for your valuable suggestion. In fact, we identified the source of heterogeneity in the subgroup analysis; the significant heterogeneity across the included studies mainly resulted from differences in the duration of sonication and vortexing.

In 4 (1-4) studies, the total duration of sonication and vortexing was ≤5 minutes, and the pooled yield was 49% (95% CI: 41–56%). No significant heterogeneity (I^2 = 31%) was observed across these studies. For studies(2, 5, 6) with a total sonication and vortexing duration of >5 minutes and ≤10 minutes, the pooled yield was 21% (95% CI: 13–29%; I^2 = 31%). Nguyen et al. sonicated explanted endoprostheses for 30 minutes, with which the yield of sonication fluid culture was only 5% (7). In these subgroup analyses, no significant heterogeneity was identified. I^2=31% (<50%) indicated insignificant heterogeneity, which was acceptable for a meta-analysis.

Subgroup analysis revealed that the significant heterogeneity seen across the included studies mainly resulted from differences in the duration of sonication and

vortexing. A total duration of more than 5 minutes will significantly decrease the yield of sonication fluid culture. This may be related to the reduced viability or even destruction of detached bacteria. The use of an ultrasound bath for 1–5 minutes at 40 kHz may yield the optimal diagnostic performance for bacterial colonization (49%, 95% CI 41–56%). This indicated that the significant heterogeneity across included studies mainly came from the difference in duration of sonication and vortexing. Meanwhile, with the extension of the duration of sonication and vortexing, the positive rate of sonication fluid culture gradually decreased.

Meanwhile, subgroup analysis was conducted according to the type of orthopedic devices with presumed aseptic loosening. Two(1, 2) studies involving a total of 114 patients reported the yield of sonication fluid culture for detecting microorganisms from spinal fusion instrumentations. No significant heterogeneity ($I^2 = 0\%$) was found between these studies, and the pooled yield was 42% (95% CI: 33–51%). A further 6(3-8) studies involving a total of 307 patients reported the yield of sonication fluid culture in artificial joints. Significant heterogeneity ($I^2 = 91\%$) was observed across these studies, and the pooled yield for detecting the presence of microorganisms was 29% (95% CI: 13–47%)

8、Comment: I agree with the limitations that you have found, and I believe that the conclusion does not reflect the discussion or the data obtained

Response: Thank you for your valuable suggestion.

We have rewritten the conclusion in the revised manuscript. We believe it now reflects the Discussion and the data obtained.

“Colonization by low-virulent microorganisms may exist in many patients with clinically presumed aseptic loosening of orthopedic devices. A sonication bath of explanted orthopedic devices for 1–5 minutes at 4 kHz could act as a powerful diagnostic tool to detect bacterial colonization. However, organism colonization detected by sonication fluid culture may not influence the outcome of one-stage revision surgery for presumed aseptic loosening. More research is required to determine whether sonication fluid culture should be incorporated into the routine treatment strategy for orthopedic device loosening.” These points could be proved by evidences listed in the revised manuscript.

“Colonization by low-virulent microorganisms may exist in many patients with clinically presumed aseptic loosening of orthopedic devices.” Sonication fluid culture, which has already been widely used for diagnosing periprosthetic joint infection, could produce a positive rate in 32% of patients with presumed aseptic loosening of orthopedic devices, which is superior to periprosthetic soft tissue culture and aspirated joint fluid culture.

“A sonication bath of explanted orthopedic devices for 1–5 minutes at 4 kHz could act as a powerful diagnostic tool to detect bacterial colonization.” Subgroup analysis revealed that the significant heterogeneity seen across the included studies mainly resulted from differences in the duration of sonication and vortexing. A total duration of more than 5 minutes will significantly decrease the yield of sonication fluid culture. This may be related to the reduced viability or even destruction of detached bacteria. The use of an ultrasound bath for 1–5 minutes at 40 kHz may yield the optimal diagnostic performance for bacterial colonization (49%, 95% CI 41–56%). This indicated that the significant heterogeneity across included studies mainly came

from the difference in duration of sonication and vortexing. Meanwhile, with the extension of the duration of sonication and vortexing, the positive rate of sonication fluid culture gradually decreased.

"However, organism colonization detected by sonication fluid culture may not influence the outcome of one-stage revision surgery for presumed aseptic loosening. More research is required to determine whether sonication fluid culture should be incorporated into the routine treatment strategy for orthopedic device loosening." The short- or long-term outcomes of revision surgery were reported in three(5, 7, 8) studies. Kemthorne et al.(8) applied one-stage revisions to 8 cases with positive microbiological outcomes of sonication fluid culture and 45 cases with negative outcomes. All patients eventually recovered without recurrent infection or repeated loosening of the implants. Similar results of one-stage revision were obtained by Nguyen et al.(7) and Bereza et al.(5). Bereza et al.(5) also evaluated patients with two-stage revision. Eventually, failures, characterized by prolonged antibiotic therapy or incision healing across an average of 2 years of follow-up, was found to be more frequent in the positive sonication fluid culture group (2/5, 40%) than in the negative sonication fluid culture groups (2/7, 28.57%); however, the difference was not statistically significant ($\chi 2=0.1714$, p-value=0.68).

Reviewer B

Comment: Literature review is very poor and a lot of papers reporting on sonication treatment are missing. Please make improvements.

Response: We have revised the manuscript to improve the literature review.

Three electronic databases including PubMed，Embase and the Cochrane central library were searched again by using a combination of two keywords including “sonication” and “loosening”. We knew that a lot of original studies and systematic reviews reporting the use of sonication in implant-related infections(10-12). However, To our knowledge, no systematic reviews have previously been conducted to investigate the application of sonication fluid culture in presumed aseptic loosening of orthopedic devices. This is the first systematic review and meta-analysis on this topic. In the current study, we compared the test power of sonication fluid culture with intra-operative culture and aspirated fluid culture and evaluated the clinical characteristics and outcomes of this unique patient cohort.

Eligible studies were peer-reviewed publications containing empiric data on the yield of sonication fluid culture for human patients whose orthopedic devices (artificial joints, osteo-synthetic materials, pedicle screws) were removed for loosening without evidence of infection such as sinus tract, highly elevated serum biomarkers, local swelling and fever. Therefore, only eight articles were considered to satisfy all the inclusion criteria for the quantitative analysis and one was included in qualitative analysis.

We admit that the small number of included studies might hinder the generalization of the obtained results and conclusion. This point has been emphasized in the Discussion of the revised manuscript. More large-scale prospective studies are needed to verify the findings of the current study.

Also, we have further scrutinized the included studies, summarized the useful information, and revised the original manuscript. Moreover, the article has been polished by native English speakers.

We believe that these actions have improved the quality of our literature review.

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