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Original article

Is an isolated positive sonication fluid culture in revision arthroplasties clinically relevant?

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ABSTRACT

Objectives: The aim of this study was to investigate the clinical relevance of an isolated positive sonication fluid culture (SFC) in patients who underwent revision surgery of a prosthetic joint. We hypothesized that cases with a positive SFC have a higher rate of infection during follow-up compared with controls with a negative SFC.

Methods: This retrospective multicentre observational study was performed within the European Study Group of Implant-Associated Infections. All patients who underwent revision surgery of a prosthetic joint between 2013 and 2019 and had a minimum follow-up of 1 year were included. Patients with positive tissue cultures or synovial fluid cultures were excluded from the study.

Results: A total of 95 cases (positive SFC) and 201 controls (negative SFC) were included. Infection during follow-up occurred in 12 of 95 cases (12.6%) versus 14 of 201 controls (7.0%) ($p = 0.125$). In all, 79.8% of cases were with treated with antibiotics (76/95). Of the non-treated cases, 89% (17/19) had a positive SFC with a low virulent microorganism. When solely analysing patients who were not treated with antibiotics, 16% of the cases (3/19) had an infection during follow-up versus 5% of the controls (9/173) ($p = 0.08$).

Discussion: Although not statistically significant, infections were almost twice as frequent in patients with an isolated positive SFC. These findings require further exploration in larger trials and to conclude

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about the potential benefit of antibiotic treatment in these cases. **Christien Rondaan, Clin Microbiol Infect 2023;29:1431**

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Introduction

Even though many improvements have been made in optimizing culture yield in recent decades, culture-negative peri-prosthetic joint infections (PJIs) remain a significant part of the PJI spectrum with an estimated incidence of around 10% [1]. Isolating the causative microorganism in these cases is critical, because an unrecognized infection and subsequent inadequate antibiotic treatment results in prosthesis failure in the long term. Prosthetic joints that are revised because of presumed aseptic loosening (i.e. with negative intraoperative tissue cultures) have a worse outcome when other minor criteria of infection are present [2], suggesting that isolation of the causative microorganism has failed. To minimize these unrecognized chronic PJIs, more sensitive criteria to diagnose PJI have been developed by the European Bone and Joint Infection Society (EBJIS) in which sonication fluid culture (SFC) plays a more prominent role compared with other available diagnostic criteria [3]. Sonication of retrieved implants has shown to improve sensitivity in the diagnosis of PJI [4] and it has been demonstrated that around 10% of sonication fluids are culture positive in tissue culture-negative revisions [5,6]. Therefore, according to the EBJIS criteria, a positive SFC (>50 CFU/mL of any microorganism for uncentrifuged samples) should be considered as a confirmatory criterion to diagnose a PJI. However, limited data are available about the clinical meaning of positive culture results in exclusively sonication fluid, and whether infection or prosthesis failure during follow-up will occur when these detected microorganisms are left untreated.

We hypothesized that those patients undergoing revision surgery of a prosthetic joint in whom microorganisms are exclusively cultured in sonication fluid have a higher rate of infection and prosthesis failure during follow-up compared with those with negative cultures.

Methods

Study population

This retrospective multicentre observational study was performed within the European Study Group of Implant-Associated Infections. Patients (cases) were included in the study if they underwent a one- or two-stage revision surgery of a prosthetic joint and had a positive culture of sonication fluid but negative intraoperative tissue and synovial fluid cultures. A positive SFC was defined according to the cut-off values stated in the EBJIS diagnostic criteria for PJI (>50 CFU/mL of any microorganism for uncentrifuged samples) [3]. A microorganism was considered virulent in case of *Staphylococcus aureus*, *S. lugdunensis*, enterococci, *Candida*, streptococci and Gram-negative rods. Coagulase negative staphylococci (other than *S. lugdunensis*), Corynebacteria and Gram-positive anaerobes were considered as low virulent. In addition, control patients were included. These controls also underwent one- or two-stage revision surgery but had next to negative tissue cultures and synovial fluid cultures, also a negative SFC. For both groups a minimum follow-up of 1 year was required for inclusion unless the endpoints already occurred within the first year after revision.

Exclusion criteria: (a) patients who underwent revision surgery in case of an acute PJI (e.g. patients who failed after surgical debridement and/or if revision surgery was performed as a first surgical approach for an acute PJI) and/or (b) patients in whom <3 tissue cultures were obtained and/or (c) patients with an insufficient follow-up and/or (d) patients with a sinus tract and/or (e) patients who received antibiotic treatment within 2 weeks before revision surgery.

The primary endpoint was infection during the follow-up period. Infection was defined according to the EBJIS diagnostic criteria [3], see text box below for confirmatory criteria. The secondary endpoint was prosthesis failure during follow-up, defined as the need for prosthesis removal for any cause.

Data on the initiation of antimicrobial treatment after revision surgery were collected. If a patient was diagnosed with infection according to the treating physician, the patient received at least 6 weeks of antimicrobial treatment.

Ethical approval was obtained by each participating centre according to local rules and obligations.

EBJIS diagnostic criteria for PJI (infection is confirmed with any positive finding)	
Clinical features	Sinus tract with evidence of communication to the joint or visualization of the prosthesis
Synovial fluid cytological analysis	>3000 cells/μL
Synovial fluid biomarkers	>80% polymorphic neutrophils
Microbiology	Alpha defensin-positive immunoassay or lateral-flow assay
Histology	≥2 positive samples with the same microorganism
	>50 CFU/mL sonication fluid of any organism
	Presence of ≥5 neutrophils in ≥high power field
	Presence of visible microorganisms

Statistical analysis

The difference in the distribution of continuous variables in more than two groups was assessed by using a Kruskal–Wallis test. The difference in distribution between two groups was performed using the Mann–Whitney U test for continuous and Fisher's exact test for binary variables. A Kaplan–Meier survival analysis was performed to assess the occurrence of infection during the follow-up period. The resulting curves were compared using the log-rank method. A multivariate analysis was performed to identify predictors of infection during follow-up using the enter method. For this analysis, those variables with a p value < 0.1 according to the univariate analysis were included in the multivariate model. Missing values were not included in the analysis. Statistical analysis was performed using IBM SPSS Statistics 28 (IBM). P values ≤ 0.05 (two-sided) were considered significant.

Results

Study population

In total, 95 cases (patients with an isolated positive SFC) and 201 controls (not having any positive cultures including a negative SFC) from 11 hospitals and from 5 different countries were included. Revision surgery took place between January 2013 and November

Table 1
Characteristics of cases and controls

	Cases (n = 95)	Controls (n = 201)	p
Age, median (range) (y)	69 (22–88)	68 (34–90)	0.922
Female gender (n)	54.7% (52/95)	62.7% (126/201)	0.205
Inflammatory arthritis (n)	7.4% (7/95)	6.0% (12/201)	0.621
Joint (n)			0.102 ^b
Hip	58.9% (66)	49.3% (99/201)	
Knee	38.9% (37)	50.2% (101/201)	
Shoulder	2.1% (2)	0.5% (1/201)	
Type of prosthesis (n)			0.770
Primary	77.9% (74)	75.6% (152/201)	
Revised	22.1% (21)	24.4% (49/201)	
Type of revision (n)			0.002
One-stage	66.3% (63/95)	83.1% (167/201)	
Two-stage	33.7% (32/95)	16.9% (34/201)	
Time from implantation to revision ^a , median (range) (d)	1245 (90–12 022)	1568 (500–10 205)	0.763
C-reactive protein (n)			<0.001
<5 mg/L	23.8% (20/84)	57.3% (90/157)	
5–10 mg/L	21.4% (18/84)	19.1% (30/157)	
>10 mg/L	54.8% (46/84)	23.6% (37/157)	
Erythrocyte sedimentation rate (mm/h) median (range) [missing]	30 (6–117) [26]	19.5 (1–120) [77]	<0.001
Histology positive for infection	50% (32/64)	8.1% (8/99)	<0.001
Histology positive for metallosis	23.4% (15/64)	15.8% (15/95)	0.23
Synovial fluid			
Leukocyte count, median (range) (cells/μL) [missing]	3450 (30–17 150) [58]	1000 (10–16 260) [145]	<0.001
Polymorphonuclear cells, median (range) [missing]	80 (23–95) [59]	50 (12–86) [147]	—
Loosening on X-ray (n)	64.2% (61/95)	65.7% (132/201)	0.597
Treated as infection (n)	79.8% (75/94)	14.1% (28/199)	<0.001
Follow-up complicated by infection (n)	12.6% (12/95)	7.0% (14/201)	0.125

IQR, interquartile range.

Bold: P values < 0.05.

^a Missing data on implantation date for 1 case and 11 controls.^b Patients with a shoulder prosthesis were excluded from the statistical analysis.

2019. Characteristics of cases and controls are shown in Table 1. Cases underwent a two-stage revision more often and had higher levels of inflammatory parameters in serum and synovial fluid compared with controls. Cases were treated for infection with antimicrobials more often than controls (79.8% [76/95] versus 14.1% [28/201], respectively, $p < 0.001$). Treated cases had a higher suspicion of a PJI because they underwent a two-stage exchange more often and less frequently had an alternative explanation for implant dysfunction (Table S1).

Cultured microorganisms from the cases are shown in Table 2. A virulent microorganism was cultured in 37.3% (28/75) of the treated cases and in 15.8% (3/19) of the untreated cases (see Material and method section for the definition). In all, 49.5% (47/95) of cases with an isolated positive SFC also had other confirmatory criteria for infection present (i.e. positive histology and/or a positive synovial fluid leukocyte count) [3].

For patients who did not experienced infection or prosthesis failure during the follow-up period, the median follow-up time since revision surgery was 1158 days (interquartile range 883–1590).

Infection and prosthesis failure during follow-up

Infection during follow-up occurred in 12 of 95 cases (12.6%) versus 14 of 201 controls (7.0%) ($p = 0.125$). A Kaplan–Meier survival analysis is shown in Fig. 1. No statistically significant difference in infection distribution among the groups could be determined ($\chi^2 = 2.450$, $p = 0.118$). Infection during follow-up was 12.2% [5/41] for cases who also had other confirmatory criteria for infection present [3] versus 14.3% [3/21] for those who did not ($p = 0.55$). A multivariate regression analysis is shown in Table S2. The only independent predictor for infection during follow-up was having a revision prosthesis that needed to be revised (OR 4.99 [95% CI 2.09–11.91], $p < 0.001$).

We performed a sub-analysis for cases and controls not treated with antimicrobials after revision surgery. The majority of cases and controls underwent a one-stage revision (94.7% [18/19] versus 92.4% [158/171], respectively, $p = 0.58$). Three of 19 cases (15.8%) that were not treated with antimicrobials experienced infection during follow-up versus 8 out of 171 controls (4.7%) ($p = 0.084$). A Kaplan–Meier survival analysis (Fig. 2) demonstrated a statistically significant difference in infection during follow-up among the untreated cases and controls ($\chi^2 = 3.984$, $p = 0.046$).

Prosthesis failure during follow-up because of any cause (including infection) was 11.7% (8/95) in cases versus 8.0% (16/201) in controls ($p = 0.30$).

Table 2
Cultured microorganisms in sonication fluid (n = 95)

	n (%)
Coagulase negative staphylococci	48 (50.5%)
<i>Staphylococcus epidermidis</i>	24
<i>Staphylococcus hominis</i>	6
<i>Staphylococcus capitis</i>	3
<i>Staphylococcus haemolyticus</i>	3
<i>Staphylococcus xylosus</i>	2
<i>Staphylococcus lentus</i>	2
<i>Staphylococcus mutans</i>	1
<i>Staphylococcus caprae</i>	1
<i>Staphylococcus cohnii</i>	1
<i>Staphylococcus saprophyticus</i>	1
<i>Staphylococcus lugdunensis</i>	1
Not specified	3
<i>Staphylococcus aureus</i>	5 (5.3%)
Streptococcus species	7 (7.4%)
<i>Enterococcus faecalis</i>	7 (7.4%)
Cutibacterium species	13 (13.7%)
Gram-negative rods	11 (11.6%)
Other	4 (4.1%)

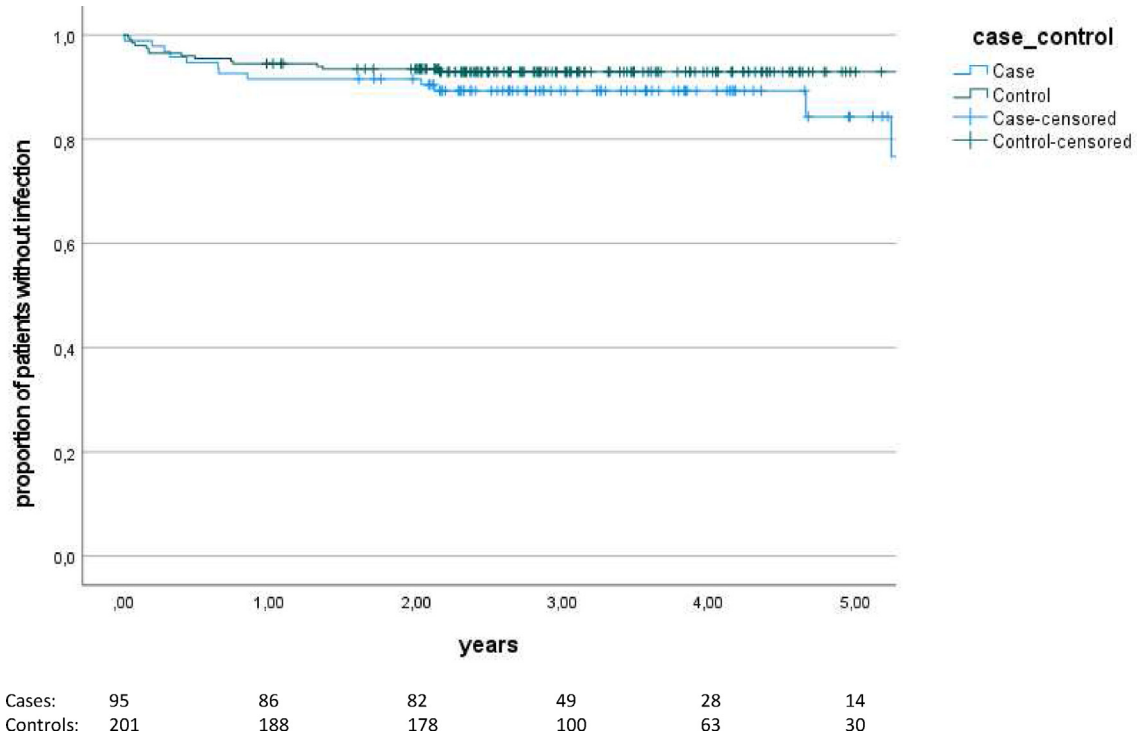


Fig. 1. Survival distribution (Kaplan-Meier) plot for 95 cases with a positive sonication fluid culture and 201 controls with a negative sonication fluid culture.

Evaluation of infection during follow-up among untreated cases

Of the 19 positive SFC cases who were not treated as having an infection at the time of prosthesis revision, 3 patients experienced infection during the follow-up period. Only for 1 case, the microorganism cultured during follow-up was the same as the initial SFC (*Streptococcus agalactiae*).

From the remaining 16 cases that were not treated as having an infection and did not experience infection during the follow-up period, the microorganisms cultured in sonication fluid were coagulase-negative staphylococci (11), Cutibacterium species (2), enterococcus species (1), Gram-negative rods (1) and Gram-positive rods other than Cutibacterium species (1).

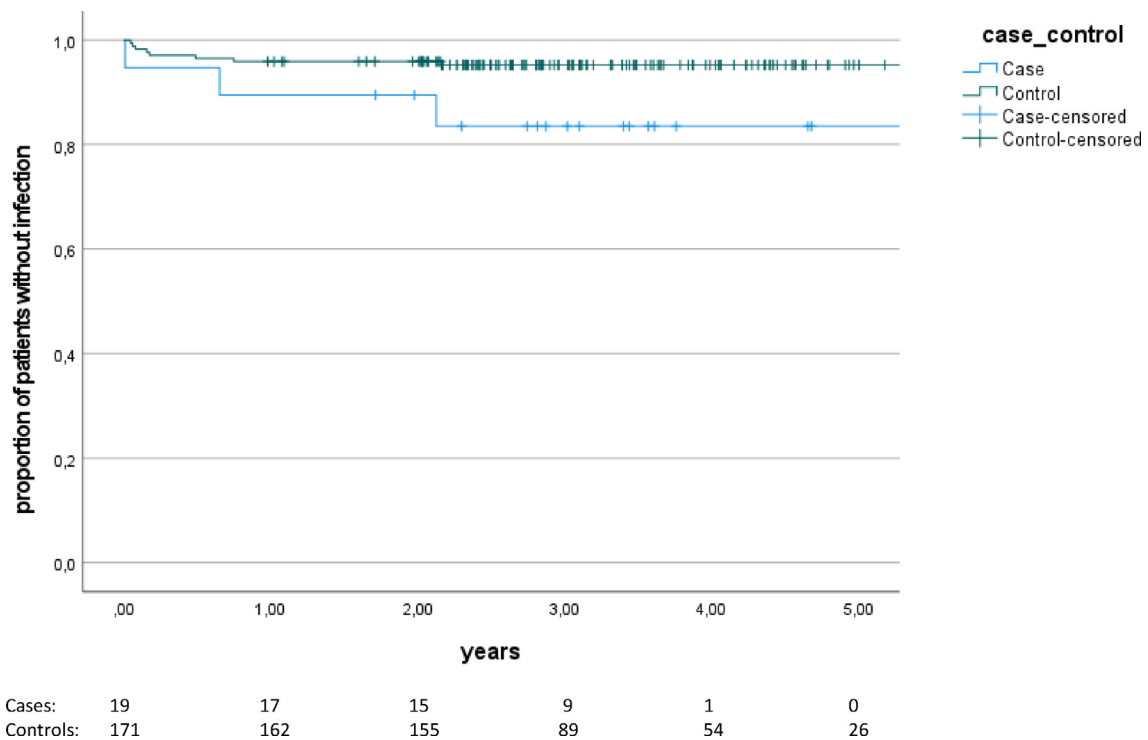


Fig. 2. Survival distribution (Kaplan-Meier) plot including cases (n = 19) and controls (n = 171) not treated with antimicrobial therapy.

Discussion

In the current multicentre retrospective case-control study, we selected cases with an isolated positive SFC during revision surgery and compared their outcomes in terms of infection and prosthesis failure during follow-up with control patients with a negative SFC. Follow-up time was until the occurrence of failure, or at least 1 year for other patients (median follow-up time 1158 days with an interquartile range of 883–1590 days).

An almost two times higher occurrence of infection during follow-up was observed between cases and controls. Cases that were left untreated did had a 11% higher infection rate during follow-up compared with control patients, although this was only caused by the same microorganism in one case (an untreated *Streptococcus agalactiae*) [3]. We are not able to conclude whether antimicrobial treatment is needed in all cases with an isolated SFC, because the vast majority of untreated cases had low virulent microorganisms in their SFC and most of them had an uneventful follow-up. Future prospective studies are needed in which all other (non-microbiological) diagnostic criteria for infection are collected to determine in which cases the isolation of low virulent microorganisms in SFC are clinically relevant.

Previous studies have demonstrated that sonicating prosthetic implants have multiple benefits, especially when patients have been pre-treated with antimicrobial treatment [7]. When pre-treated, culture yield increases with approximately 30% when using sonication. Sonication also reduces the time of positivity of cultures and, therefore, provides a more rapid infection diagnosis [8–10]. Ribeiro et al. [11] recently demonstrated that a positive SFC may aid in infection diagnosis in inconclusive cases and overall increases the sensitivity when compared with tissue cultures. The EBJS is the first who considers a single positive SFC (>50 CFU/mL, when no centrifugation is applied) as confirmatory criterion for infection diagnosis [3].

The clinical importance of only one positive culture in tissue samples has been studied, but the results of these investigations are controversial [12–14].

Our study should be viewed in the light of certain limitations. Apart from its retrospective study design, the most important limitation is that the number of cases with a positive SFC that were left untreated were limited ($n = 19$). Although the infection rate was higher in those that did not receive antimicrobial treatment, most untreated cases had an uneventful follow-up (16 of 19) and some of the infections did not match the initial positive SFC. On the basis of these findings one can question whether antimicrobial treatment is really needed. However, it should be noted that most of the cultured microorganisms that were left untreated were low virulent microorganisms (mainly coagulase-negative staphylococci). Unfortunately, although all cases had a positive SFC of at least 50 CFU/mL, the exact number of CFU was not documented in all cases and the standard operating procedure protocol were not available for all centres, hampering the possibility to study the microbiological significance in more detail. These important details should be addressed in future prospective studies. Most of the virulent microorganisms isolated in our study were treated. Moreover, our data suggest that the treated cases had a higher suspicion of a PJI because they underwent a two-stage exchange more often and less frequently had an alternative explanation for implant dysfunction.

In conclusion, patients undergoing revision surgery of a prosthetic joint with an isolated positive SFC have an almost two times higher reinfection rate during follow-up compared with patients without any positive culture and, therefore, should be considered treated with antibiotics. Future prospective studies in a larger cohort of patients are warranted to identify the microbiological cut-

off value in CFU/mL for different microorganisms, especially for common contaminants.

Author contributions

CR performed the statistical analyses and wrote the initial draft of the manuscript. MW-B designed the study, supervised CR in writing the manuscript and in performing the analysis. AM, R-MB, MFS, AS, VDdB, JGJ, MDDT, JGH, MJS and JE critically appraised the study protocol, collected data and reviewed and edited the manuscript.

Transparency declaration

Conflict of interest

AS received a grant from Pfizer not related to this study, consulting fees and payment or Honoraria for lectures from Pfizer, MSD, Angelini, Shionogi, Gilead, Menarini and support for attending meetings from Pfizer. JGJ received support to attend international meetings from Angelini, Pfizer and MSD. JE received a grant, payment for an expert testimony and consulting fees from BioMérieux and support for attending meetings from Angelini and Pfizer. MW-B received payment or Honoraria for lectures from BioMérieux and Zimmer Biomet. The remaining authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2023.07.018>.

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