Diagnostic Microbiology and Infectious Disease 84 (2016) 112–115

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Comparison of bacterial growth in sonication fluid cultures with periprosthetic membranes and with cultures of biopsies for diagnosing periprosthetic joint infection

Gunnar T.R. Hischebeth [a,*], Thomas M. Randau [b], Ernst Molitor [a], Matthias D. Wimmer [b], Achim Hoerauf [a], Isabelle Bekeredjian-Ding [a,c,1], Sascha Gravius [b,1]

[a] Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany
[b] Department of Orthopaedics and Trauma Surgery, University Hospital Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn, Germany
[c] Division of Microbiology, Paul-Ehrlich-Institute, Paul-Ehrlich-Str 51-59, D-63225 Langen, Germany

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 8 September 2015
Accepted 9 September 2015
Available online 12 September 2015

Keywords:
PJI
Sonication
Histopathology
Periprosthetic membranes

ABSTRACT

Total joint arthroplasty is a common operation worldwide with infection rates between 1% and 3%. In cases of suspected periprosthetic joint infection, it is very challenging to rule out the causative microorganisms. In this study, we compared the appearance of periprosthetic membranes with the microbiological results obtained from cultures of sonication fluid and the correlation between classical microbiological cultures and cultures of sonication fluid. The results confirmed a strong correlation of bacterial growth in sonication fluid cultures with bacterial growth in classical microbiological cultures. Most importantly, however, our study documented a highly significant correlation of periprosthetic membranes typical for periprosthetic joint infection (PJI) with bacterial growth in sonication fluid. Sonication fluid cultures yielded a better sensitivity than tissue cultures (72.34–60.87%). These 3 methods are useful tools in diagnosing PJIs, and even more, sonication fluid cultures should be included in the diagnostic path of PJI.

1. Introduction

Total joint arthroplasties, especially total knee and hip arthroplasties, belong to the most successful surgical procedures worldwide with great patient satisfaction and improving patient quality of life. Approximately 340,000 total hip and knee arthroplasties are performed in Germany per year (Bundesamt, 2014). Recent studies show a stable frequency of total hip arthroplasties per year accompanied by an infection rate between 1% and 3% (Lange et al., 2012; Parvizi et al., 2008; Peersman et al., 2001). Due to the demographic changes, an increase in the number of total hip and knee arthroplasties is expected in the next decades, and this will unavoidably be associated with an increase in the number of periprosthetic joint infections (PJI) (Kurtz et al., 2012).

PJI leads to difficult, cost intensive treatment, functional deficit, and long-lasting hospitalization. In the United States, medical costs for PJI rose from 320 million dollars per year in 2001 to 566 million dollars per year in 2009 (Kurtz et al., 2012) It is expected that medical treatment costs for PJI will rise to 1.62 trillion dollars per year in the year 2020 (Kurtz et al., 2012).

In cases of suspected PJI, microbiological methods represent a very important hallmark of treatment concepts (Wimmer et al., 2013). Due to this fact, it is essential to diagnose PJI early and reliable with all available methods (Friedrich et al., 2014; Randau et al., 2014). Recently, sonication was implemented as a new diagnostic method in PJI (Evangelopoulos et al., 2013; Scorzolini et al., 2014a; Trampuz et al., 2007; Tunney et al., 1998; Zhai et al., 2014). In periprosthetic knee and hip infection, it was shown that sonication has a higher sensitivity than tissue cultures (78.5% versus 60.8%) (Trampuz et al., 2007). Another important diagnostic tool is the histopathological examination of periprosthetic membranes according to the consensus classification of Morawietz et al. (2006) and Krenn et al. (2013) that occur in infected total joint arthroplasties. Notably, periprosthetic membrane types 2 and 3 according to Morawietz and Krenn are associated with PJI.

In this study, we evaluated the correlation between cultured sonication fluid results and the presence of periprosthetic membrane type 2 or type 3 in the histopathological examination.

2. Material and methods

2.1. Patient selection

Eighty patients who received an operative revision in the orthopedic department of the University Hospital Bonn due to suspected PJI or aseptic loosening of a painful total hip or knee arthroplasty between October 2012 and July 2014 were included in this retrospective study. A minimum of 3 biopsies and the removed hardware from each patient was sent for microbiological analysis. Patients were treated according

* Corresponding author. Tel.: +49-228-287-15952; fax: +49-228-287-19573.
E-mail address: hischebeth@microbiology-bonn.de (G.T.R. Hischebeth).
[1] Authors contributed equally.

http://dx.doi.org/10.1016/j.diagmicrobio.2015.09.007
0732-8893/

to the established treatment algorithm as published elsewhere, and samples were evaluated within the routine clinical diagnostics (Wimmer et al., 2013).

2.2. Sonication protocol

The explanted prostheses were transferred to the microbiological laboratory for the sonication procedure. In the operating theater, the explant was placed into a sterile plastic container (Lock & Lock; Distributor ISI, Solingen, Germany). Upon arrival, sterile saline solution was added under the laminar flow, and the specimen was vortexed for 30 seconds. Subsequently, the sonication was performed in a Bactosonic 14.2 device (Bactosonic; Bandelin, Berlin, Germany) for 5 minutes with a frequency of 40 ± 2 kHz and power density 0.22 ± 0.04 W/cm^2 followed by 30 seconds of vortexing. Sonication fluid was transferred to sterile 50-mL Falcon tubes (Becton & Dickinson, Heidelberg, Germany) and centrifuged for 15 minutes with 4200 g. After that, the supernatant was reduced to 10 mL volume in which the pellet was resuspended. The resulting homogenized sonication fluid was used for inoculation of culture media.

2.3. Microbiological cultures

Sonication fluid (0.5 mL) as well as shredded and homogenized intraoperatively collected tissue specimens was plated on Columbia agar with 5% sheep blood, MacConkey agar, chocolate agar, and sabouraud agar (all from Becton & Dickinson), while 1 mL was pipetted into thioglycolate boullion (Becton & Dickinson). Additionally, Schaedler and kanamycin/vancomycin agar plates (Becton & Dickinson) for anaerobic cultures were striked with 0.5-mL sonication fluid. Cultures were grown at 5% CO_2 and 35 °C for at least 14 days. In parallel, sonication fluid was added in PEDS medium blood culture flasks (Becton & Dickinson) and incubated in a Bactec FX blood culture system (Becton & Dickinson) for 14 days (Schafer et al., 2008).

2.4. Histopathological examination

During the surgical procedure, samples of the periprosthetic membranes from the bone-implant interface were collected and immediately fixed in 4% formalin. Samples were then embedded in paraffin and cut to sections of 5 μm. Subsequently, hematoxylin/eosin staining was performed, and periprosthetic membranes were classified by a trained histopathologist as described by Morawietz et al. (2006) and Krenn et al. (2013). This method distinguishes infection from aseptic loosening by defining four histopathological entities according to the presence of neutrophil infiltrates, granulation, and foreign-body particles.

2.5. Statistical analysis

Data was collected in Microsoft Excel 2010(Microsoft Corporation, Richmond, USA). Fisher's exact test was used for statistical analysis and performed to compare between results of sonication fluid cultures, conventional microbiological cultures, and histopathological periprosthetic membranes. Concordance between sonication fluid cultures and conventional microbiological cultures and, respectively, between sonication fluid cultures and histopathological membranes was analyzed.

3. Results

Samples from a total of 80 patients were included in the analysis. Our data show that 34 of 80 (42.5%) included patients had positive bacterial growth in sonication fluid and concomitantly had evidence for type 2 or 3 membranes in histopathological examination. Of 80 patients, 25 (31.25%) had negative results in sonication fluid culture and showed no evidence for PJI in the histopathological examination. Of 80 patients, 13 (16.25%) showed no growth in sonication fluid culture but showed signs of PJI in histopathology. Of 80 patients, 8 (10%) had positive sonication fluid culture but no signs of PJI in histopathological examination. Statistical analysis showed high concordance between the groups (Fisher's exact test, $P < 0.0001$) (Table 1).

Table 1
Cross tables with correlation between sonication fluid culture and histopathology in the upper table, between sonication fluid culture and bacterial proof in cultures of biopsiesin the middle table and correlation between cultures of biopsiesand histopathology in the lower table.

		Membrane type 2 or 3		
		Positive	Negative	Sum
Positive bacterial proof in sonication	Positive	**34**	**8**	42
	Negative	**13**	**25**	38
	Sum	47	33	80

$P < 0.0001$

		Bacterial proof in cultures of biopsies		
		Positive	Negative	Sum
Positive bacterial proof in sonication	Positive	**29**	**14**	43
	Negative	**6**	**31**	37
	Sum	35	45	80

$P < 0.0001$

		Membrane type 2 or 3		
		Positive	Negative	Sum
Positive bacterial proof in cultures of biopsies	Positive	**28**	**8**	36
	Negative	**18**	**26**	44
	Sum	46	34	80

$P < 0.0013$

In the next step, we analyzed the concordance between sonication fluid cultures and classical microbiological tissue cultures. Here, we found that 29 of 80 included patients (36.25%) showed bacterial growth in sonication fluid cultures and in classical microbiological cultures. Of 80 patients, 14 (17.5%) had a positive sonication fluid culture but remained negative in classical microbiological cultures. Of 80 patients, 6 (7.5%) yielded positive bacterial growth in classical microbiological cultures but had no signs of bacterial growth in sonication fluid cultures. Finally, 31 of 80 patients (38.75%) had no evidence for bacterial growth neither in classical microbiological nor in sonication fluid cultures. Statistical analysis showed high concordance between the groups (Fisher's exact test, $P > 0.0001$) (Table 1).

Also, shredded tissue biopsies and periprosthetic membranes were compared. This group showed that 28 of 80 patients had bacterial growth in cultures of biopsies and additionally had evidence for type 2 or 3 membranes in histopathological examination. Of 80 patients, 26 remained negative in cultures of biopsies and had no evidence for infection in histopathology. Of 80 patients, 18 showed signs for inflammation with documented type 2 or 3 membranes in histopathology but had no bacterial growth in cultures of biopsies. Of 80 patients, 8 had positive microbiological cultures of biopsies but no evidence for inflammation in histopathological examination. Even now, statistical analysis showed high concordance between the groups (Fisher's exact test, $P > 0.0013$) (Table 1).

In addition, we analyzed sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Sonication showed a sensitivity of 72.34% with a specificity of 75.75%, PPV of 80.95%, and an NPV of 65.79%. Cultures of biopsies had a sensitivity of 60.87% with a specificity of 76.47%, PPV of 77.78%, and an NPV of 59.1%.

In approximately half of all the cases (51%), we found coagulase-negative staphylococci, followed by *Staphylococcus aureus* (15%), enterococci (11%), streptococci (7%), gram-negative rods (4%), mixed infections (4%), micrococci (2%), yeast (2%), *Corynebacterium* spp. (2%), and anaerobic bacteria (2%) in sonication fluid cultures (Fig. 1).