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Multiplex PCR of sonication fluid accurately differentiates between prosthetic joint infection and aseptic failure

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Summary *Objective:* Cultures have limited sensitivity in the diagnosis of prosthetic joint infection (PJI), especially in low-grade infections. We assessed the value of multiplex PCR in differentiating PJI from aseptic failure (AF).
Methods: Included were patients in whom the joint prosthesis was removed and submitted for sonication. The resulting sonication fluid was cultured and investigated by multiplex PCR, and compared with periprosthetic tissue culture.
Results: Among 86 explanted prostheses (56 knee, 25 hip, 3 elbow and 2 shoulder prostheses), AF was diagnosed in 62 cases (72%) and PJI in 24 cases (28%). PJI was more common detected by multiplex PCR ($n = 23$, 96%) than by periprosthetic tissue ($n = 17$, 71%, $p = 0.031$) or sonication fluid culture ($n = 16$, 67%, $p = 0.016$). Among 12 patients with PJI who previously received antibiotics, periprosthetic tissue cultures were positive in 8 cases (67%), sonication fluid cultures in 6 cases (50%) and multiplex PCR in 11 cases (92%). In AF cases, periprosthetic tissue grew organisms in 11% and sonication fluid in 10%, whereas multiplex PCR detected no organisms.
Conclusions: Multiplex PCR of sonication fluid demonstrated high sensitivity (96%) and specificity (100%) for diagnosing PJI, providing good discriminative power towards AF, especially in patients previously receiving antibiotics.

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Introduction

Prosthetic joints are increasingly used in the growing population of the elderly, mainly for treatment of the degenerative joint disease and bone fractures [1]. Since the management of aseptic failure (AF) profoundly differs from the one of prosthetic joint infections (PJI), an accurate diagnosis is crucial for treatment outcome [2]. Culture of periprosthetic tissue represents the standard method for the microbiological diagnosis of PJI, but can be false-negative in up to 30% [3–5]. Sonication of removed prosthetic devices, followed by culture of sonication fluid improved the microbiological diagnosis of PJI [6]. However, a considerable number of cultures in PJI remain false-negative. These patients could be misclassified as AF and are not treated properly [5]. The reason for limited sensitivity of cultures for the diagnosis of PJI is manifold. First, implant-associated infections are typically caused by microorganisms forming biofilm on the implant surface and are not always present in the surrounding tissues [7,8]. Second, cultures may fail to detect intracellular bacteria residing in osteoblasts [9]. Third, the use of previous antimicrobial treatment may inhibit microbial growth.

Sonication was introduced to dislodge the biofilm from removed implants, which improved the sensitivity and specificity compared to periprosthetic tissues cultures [6]. Whereas several researchers have evaluated the PCR in synovial fluid or periprosthetic tissue specimens [10–12], sonication fluid was evaluated only recently [13]. In this study, we employed both novel technologies: sonication of removed prosthesis and multiplex PCR of the resulting sonication fluid, and investigated their value for accurate differentiation between PJI and AF.

Materials and methods

Study design

This cohort study was conducted in two tertiary medical care centers, Hospital del Mar (≈400 beds) and Hospital de l'Esperança (≈200 beds) in Barcelona, Spain. Both hospitals perform jointly about 900 orthopedic surgical procedures annually, including primary and revision arthroplasties. The study protocol was reviewed and approved by the institutional review board.

Study population

We prospectively included all consecutive patients aged ≥18 years hospitalized from July 2010 through July 2011 in one of the participating hospitals, in whom the joint prosthesis or part of it (such as the polyethylene inlay) was removed for any reason. Subjects were excluded, if obvious contaminations of the explanted components occurred during surgery, transport or processing of the prosthesis in the laboratory.

Study definitions

PJI was defined if at least one of the following criteria was present: (i) visible purulence of joint aspirate or surgical site (as determined by the surgeon), (ii) presence of a sinus tract (fistula) communicating with the prosthesis, (iii) acute inflammation in histopathology sections of periprosthetic tissue (as determined by pathologist), (iv) acute inflammation in preoperative joint aspirate (i.e. leukocyte count >1.7 G/l or >65% neutrophils in knee prosthesis [14] or leukocyte count >4.2 G/l or >80% neutrophils in hip prosthesis) [15], (v) growth of a pathogen in synovial fluid or periprosthetic tissue. Low-virulence microorganisms, such as coagulase-negative staphylococci (CNS), *Corynebacterium* spp., *Bacillus* spp. or *Propionibacterium acnes*, growing only in a single periprosthetic tissue specimen, were considered pathogens, if an additional non-microbiological PJI criterion was present (see above). Early postoperative infections were considered when PJI occurred within 3 months of implantation. Delayed or low-grade infections were considered when PJI occurred between 3 and 24 months after implantation. Late infections were considered when PJI occurred 2 years after prosthesis implantation. AF was defined as prosthesis failure in the absence of any of the above criteria for PJI. Previous antimicrobial therapy was defined as receiving an antibiotic for ≥24 h in the 14 days before surgery.

Preoperative joint aspirate cultures

Synovial fluid was aspirated preoperatively at the discretion of the operating surgeon. The cell count was determined by automated hematology cell counter. For culture, synovial fluid was collected in native vials. In the microbiology laboratory, 0.1 ml was inoculated on each PoliVitex (BioMérieux, Marcy L'Etoile, France) agar plates (incubated 7 days aerobically at 37 °C with 5% CO_2) and Schaedler enriched with 5% of sheep blood (BioMérieux, Marcy L'Etoile, France) agar plates (incubated 14 days anaerobically at 37 °C). In addition, 0.5 ml of synovial fluid was inoculated in thioglycolate broth (BBL™ Enriched Thioglycolate Medium with Vitamin K & Hemin, Becton Dickinson and Company, USA) and residual volume were inoculated into a BacT/ALERT (BioMérieux, Marcy L'Etoile, France) anaerobic bottle and incubated for 5 days.

Periprosthetic tissue cultures

Tissue specimens were collected in sterile vials and were individually homogenized in 0.5 ml thioglycolate broth. Tissue homogenate samples (0.5 ml) were inoculated in PoliVitex agar plates, Schaedler enriched with 5% of sheep blood agar plates and inoculated into thioglycolate broth. The aerobic cultures were incubated at 37 °C for 7 days whereas the anaerobic ones incubated for 14 days. The colonies of each distinctive morphology were identified using standard microbiological techniques.

Sonication fluid cultures

The removed prosthesis components or mobile parts were aseptically removed in the operating room and transported to the microbiology laboratory in solid polyethylene 2000 ml containers with a cylinder body, a screw top and an internal air-tight seal. Containers were previously autoclaved at 121 °C for 15 min and double packed. Sonication