Prosthetic infection: improvement of diagnostic procedures using 16S ribosomal deoxyribonucleic acid polymerase chain reaction

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Abstract

Purpose Prosthetic infection is the worst complication in joint arthroplasty. The diagnostic procedure is time consuming and in many cases unrewarding. The aim of this investigation was to raise the sensitivity of the diagnostic procedure.

Methods Altogether, 229 implants were removed from 229 patients. Complete data from 157 patients could be analysed. On explantation of the respective arthroplasty, tissue was removed, puncture fluid aspirated and biofilm scratched from the implant surface with a surgical knife. Specimens were investigated with conventional culture methods and with 16S ribosomal DNA (rDNA) polymerase chain reaction (PCR) and sequencing. **Results** In 123 cases, no pathogen could be identified by routine culture methods. In three of these culture-negative cases, bacteria could be identified with 16S rDNA sequencing of the removed biofilm. In 34 cases, bacteria could be identified with culture methods. In two of these cases, sequencing detected additional pathogens.

Conclusions The process of 16S ribosomal deoxyribonucleic acid polymerase chain reaction (rDNA PCR) and sequencing of biofilm removed from the explanted prosthesis is an important addition to conventional culture methods in prosthetic joint infection. Polymerase chain reaction detects additional pathogens and improves diagnostic sensitivity. The examination of tissue, puncture fluid and biofilm should be performed in cases of prosthesis loosening and explantation.

Keywords Prosthetic infection · Diagnosis using 16S rDNA PCR · Biofilm

Introduction

Prosthesis infection is the worst complication in joint arthroplasty. The diagnostic procedure is laborious, time consuming and in many cases unrewarding. However, sometimes the reason for aseptic loosening is a low-grade infection, which is more difficult to handle [1]. Infection rates are 1.7 % in total hip arthroplasty (THA) and 2.5 % in total knee arthroplasty (TKA). In 1972, Charnley reported rates of 3.8 % and more [2]. In Germany, more than 350,000 primary arthroplasties were implanted in 2008 and every year about 7,000 new patients experience prosthetic infection [3]. This figure emphasises the economic need for fast and reliable diagnosis and rapid treatment. Treating infected joint replacements is extremely expensive in view of the long hospitalisation period and high treatment costs. Treating low-grade prosthetic infections is difficult, as there are many therapeutic approaches but no gold standard. Identifying prosthetic infection by polymerase chain reaction (PCR) of biofilm removed from the prosthetic surface using ultrasound is an important addition to the diagnostics of infection, as Trampuz et al. showed in 2003 [4]. The isolation of biofilm using ultrasound is a good diagnostic method. However, the sensitive methods for transporting and

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handling the explants and the associated high costs render this method impractical for use in community hospitals [5, 6]. Not every removal of an infected orthopedic implant is performed at a high-level orthopaedic centre or centre for septic surgery where the ultrasound facilities are present. In addition, there is a high risk of contamination and misdiagnosis [5]. A simple and safe method is therefore needed to improve the diagnosis of bacterial pathogens in orthopaedic implant infections, especially when conventional methods show (false) negative results, so that the patient can receive optimal antibiotic treatment.

We report the results of a prospective clinical trial using 16S ribosomal DNA (rDNA) PCR and sequencing of biofilm removed from prosthetic surfaces by scraping. The aim of this study was to discover whether PCR of the biofilm can be used to detect bacterial DNA and perhaps increase the sensitivity of the entire diagnostic procedure.

Patients and methods

Between July 2006 and December 2007, 229 arthroplasties (hip, knee, shoulder, elbow, radial head, finger joint, ankle joint) had to be removed from 229 patients due to signs of septic or aseptic loosening. The operations took place at the Department of Orthopaedics and Trauma, University of Heidelberg, Germany. A patient was diagnosed with septic loosening if there were clinical signs of infection such as fever, pain and redness, signs of inflammation in the blood count, intra-operative purulence or purulent puncture fluid. No patient had been treated with antibiotics within the seven days prior to surgery. Analysis of all patient data showed that only 157 patients had a complete series of diagnostic specimens as defined in our study protocol.

Table 1 Explant characteristics

Characteristics	Aseptic loosening	Infection	Total
Age (years)			
Median	66.6	65.9	66.3
Range	20–87	34–94	20–94
Sex [no. (% per group/total)]			
Female	72 (82.76/45.86)	15 (17.24/9.55)	87 (55.41)
Male	47 (67.14/29.94)	23 (32.86/14.65)	70 (44.59)
Site of arthroplasty			
Hip	64	27	91
Knee	39	10	49
Shoulder	9	0	9
Ankle joint	1	1	2
Thumb	2	0	2
Finger	2	0	2
Elbow	1	0	1
Unicondylar knee	1	0	1

We explanted 91 THA, 49 TKA, nine total shoulder arthroplasties, two ankle-joint arthroplasties, two thumb-joint arthroplasties, two finger-joint arthroplasties, one elbow arthroplasty and one unicondylar knee arthroplasty (Table 1). Reasons for arthroplasty were osteoarthritis and rheumatoid arthritis. Using a sterile knife, we intraoperatively removed three to five tissue specimens of periarticular synovial, puncture fluid (at least one cubic centimetre per specimen) of the joint and biofilm from the surface of all components. This was performed on a separate surgical table that had been sterilised, and the samples were stored in a sterile box with two millilitres sterile Ringer's solution (Figs. 1–3). Specimens were smaller only for finger and thumb joints. All specimens were processed on the day of retrieval at the Department for Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg, Germany.

On arrival at the laboratory, all tissue specimens were ground and cultured in thioglycolate broth and on Columbia agar 5 % sheep blood, chocolate agar, MacConkey agar and for anaerobic culture Schaedler and kanamycin–vancomycin agar. Plates and broth were cultured at 36 °C with 5 % carbon dioxide (CO₂) for a maximum of five days. A Gram stain was also performed. Removed biofilm was examined via PCR using primers for 16S rDNA and the 18S–28S internal transcription spacer (ITS) region to detect bacterial and fungal

Fig. 1 Scratching from the surface of a femoral stem

Fig. 2 Scratching from the cement of a bicondylar knee surface replacement

pathogens, respectively. Initial DNA preparation was performed using Qiagen QIAmp DNA Mini Kit. The presence of PCR products was checked with a 2 % agarose gel. Positive samples were purified with Qiagen PCR Purification Kit and sent to GATC Biotech, Germany, for sequencing. Sequences were edited with ChromasPro software and checked with BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) or BiBi (<http://pbil.univ-lyon1.fr/bibi/>). Multiple sequences were analysed using RipSeq software (<https://www.ripseq.com/login.aspx>).

Joint puncture was performed under sterile conditions according to guidelines for joint puncture and injections of the German Society of Orthopaedic and Trauma Surgery. No local anaesthesia was used. Two millilitres of puncture fluid were inoculated into a BD BACTEC™-PEDS-PLUS/F blood-culture bottle (Fa. Becton Dickinson GmbH, Heidelberg, Germany). These bottles are optimised for inoculation volumes of one to three. Blood-culture bottles were incubated in a BD BACTEC™ 9240 (Fa. Becton Dickinson) for up to seven days. Positive bottles were subcultured onto Columbia

Fig. 3 Sterile box with 2 ml fluid for specimen transportation

agar 5 % sheep blood, chocolate agar and MacConkey agar and incubated at 36 °C and 5–7 % CO₂. A Gram stain was also performed.

Approval from the competent Ethics Committee was obtained for anonymous data analysis. After data collection and analysis, the descriptive statistics were performed using SPSS® version 16.0 for Windows (SPSS Inc. Chicago, IL, USA).

Results

No infectious agent could be identified in the tissue and puncture fluids of 123 patients; however, in three of those cases, bacteria were found with 16S rDNA PCR of the removed biofilm. In the remaining 34 cases, bacteria were found by culture methods: In two of those cases, PCR detected additional bacteria. Twenty-nine of the 34 patients showed infection of the prosthesis with evidence of pain, purulence or strong signs of inflammation in the blood. Five patients had positive result without signs of inflammation in the blood. No patient with the clinical diagnosis of an infection had a negative microbiological result. Bacteria found were *Staphylococcus epidermidis* (12 cases), *S. aureus* (nine cases), *Enterococcus faecalis* (seven cases), *S. capitis* (two cases), *S. lugdunensis* (two cases), *Escherichia coli* (two cases), *Klebsiella pneumoniae* (two cases), coagulase-negative staphylococci (four cases), *S. hominis* (one case), *Enterococcus faecium* (one case), beta-hemolytic streptococci group B (one case), *Pseudomonas aeruginosa* (one case), *Corynebacterium glaucum* (one case), *Corynebacterium* spp. (one case), *Bacteroides thetaiotaomicron* (one case), *Bacteroides* sp (one case), *Propionibacterium acnes* (one case) and *Candida parapsilosis* (one case) (Table 2).

Of the 37 patients with evidence of bacterial infection, 26 had had THA, ten a TKA and one had an ankle joint arthroplasty. No bacteria could be detected in the removed shoulder- or thumb-joint arthroplasties. More than one pathogen was found with conventional microbiological methods in six patients with THA and two with TKA. With PCR, additional bacteria were detected in two cases after THA. In one case after removal of an ankle-joint arthroplasty and two after removal of THA, bacteria only were detected by PCR.

Looking at the patients' records, we detected bacteria in all 11 patients who had a positive microbiological culture at another institution before surgery. However, using PCR, we found additional bacteria in two cases. Twenty-nine patients showed clinical signs of infection with criteria mentioned above. If these 29 are taken as the "genuinely" infected, sensitivity in detecting pathogens from tissue using culture methods is 85.3 % and specificity 96.1 %. PCR sensitivity is 80.1 % and specificity 94.5 %. In this study, no patient with clinical signs of infection had a negative result using PCR. However, in three patients, one with clinical signs of infection, only PCR of the biofilm showed the pathogen.

Table 2 Results of conventional microbiological methods and 16S ribosomal DNR polymerase chain reaction (PCR) analysis

Sex	Conventional	PCR of biofilm	Joint	Clinical signs of infection
Female	<i>Staphylococcus lugdunensis</i>	<i>S. lugdunensis</i>	Hip	Yes
Female	<i>S. lugdunensis</i>	<i>S. lugdunensis</i>	Knee	Yes
Male	<i>S. epidermidis</i> , <i>Escherichia faecalis</i> , <i>Corynebacterium</i> spp.	<i>S. epidermidis</i> , <i>E. faecalis</i> , <i>Corynebacterium</i> spp.	Hip	Yes
Male	<i>S. epidermidis</i> , <i>E. faecalis</i>	<i>S. epidermidis</i> , <i>E. faecalis</i>	Knee	Yes
Male	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Male	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Male	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Male	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Male	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Male	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Female	<i>S. epidermidis</i>	<i>S. epidermidis</i> , <i>Klebsiella pneumoniae</i>	Hip	Yes
Female	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Female	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Hip	Yes
Female	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Knee	Yes
Male	<i>S. capitis/caprae</i>	<i>S. capitis/caprae</i>	Knee	Yes
Male	<i>S. capitis</i> spp., coagulase-negative <i>S. capitis</i>	<i>S. capitis</i> spp. <i>S. capiti</i> , <i>Propionibacterium</i> <i>acnes</i>	Hip	Yes
Male	<i>S. aureus</i> , beta-hemolytic <i>Streptococcus</i> B	<i>S. aureus</i> , beta-hemolytic <i>Streptococcus</i> B	Knee	Yes
Male	<i>S. aureus</i>	<i>S. aureus</i>	Hip	Yes
Male	<i>S. aureus</i>	<i>S. aureus</i>	Hip	No
Female	<i>S. aureus</i>	<i>S. aureus</i>	Hip	Yes
Male	<i>S. aureus</i>	<i>S. aureus</i>	Knee	No
Male	<i>S. aureus</i>	<i>S. aureus</i>	Knee	Yes
Male	<i>S. aureus</i>	<i>S. aureus</i>	Knee	Yes
Female	<i>S. aureus</i>	<i>S. aureus</i>	Knee	Yes
Female	<i>Pseudomonas aeruginosa</i>	<i>P. aeruginosa</i>	Knee	Yes
Female	Coagulase-negative staphylococci	Coagulase-negative staphylococci	Hip	Yes
Female	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Hip	Yes
Female	<i>E. faecalis</i> , coagulase-negative staphylococci, <i>E. coli</i> , ESBL	<i>E. faecalis</i> , coagulase-negative staphylococci, <i>E. coli</i> , ESBL	Hip	Yes
Female	<i>E. faecalis</i> , <i>E. faecium</i> , VRE	<i>E. faecalis</i> , <i>E. faecium</i> , VRE	Hip	Yes
Male	<i>E. faecalis</i> , <i>Bacteroides thetaiotaomicron</i> , <i>Bacteroides</i> spp.	<i>E. faecalis</i> , <i>B. thetaiotaomicron</i> , <i>Bacteroides</i> spp	Hip	Yes
Male	<i>E. faecalis</i>	<i>E. faecalis</i>	Hip	Yes
Male	<i>E. faecalis</i>	<i>E. faecalis</i>	Hip	Yes
Female	<i>E. coli</i>	<i>E. coli</i>	Hip	No

Table 2 (continued)

Sex	Conventional	PCR of biofilm	Joint	Clinical signs of infection
Male	<i>C. glaucum</i>	<i>C. glaucum</i>	Hip	Yes
Male	<i>Candida parapsilosis</i> , coagulase-negative staphylococci	<i>C. parapsilosis</i> , coagulase-negative staphylococci	Hip	Yes
Male	0	Coagulase-negative staphylococci	Hip	No
Male	0	<i>S. aureus</i>	Hip	Yes
Male	0	<i>S. epidermidis</i> and <i>S. hominis</i>	Ankle	No

ESBL extended-spectrum beta lactamases, *VRE* vancomycin-resistant enterococci

Discussion

This study shows that the 16S rDNA PCR of biofilm, removed by scratching from prosthetic surfaces, is a valuable addition to conventional culture methods for identifying the infectious agent in prosthetic joint infection: Polymerase chain reaction is able to detect additional bacteria. The advantage of the technique presented here is that it is a simple procedure that does not require expensive ultrasound baths or special transport media, as do other methods described in the literature [5–11]. With a fast and correct diagnosis, treatment outcome is clearly better and costs lower [12–14]. Results also show that diagnostics of tissue, liquid and biofilm should be performed in prosthetic joint explantation surgery to ensure complete and easy methods of bacteria identification [13, 15, 16]. A fast and reliable diagnosis of the infection, together with the appropriate therapy, is vital for a good prognosis. Microbiological isolation of the pathogen and identification from puncture fluid are essential for correct diagnosis. [6, 12, 17, 18]

Polymerase chain reaction has some important limitations regarding sensitivity and detection limit. This is due to the ubiquitous contamination of reagents with bacterial or fungal DNA during manufacture. However, DNA-free commercial reagents are not yet available. The method used in this study has a limited sensitivity but nevertheless falls within the scope of values published in the literature.

The period between specimen isolation and processing in the laboratory should be as short as possible to ensure survival of pathogens that are difficult to culture. This period should not exceed six hours, which is difficult to achieve in view of the centralisation of microbiological laboratories. Equally, transport conditions such as ambient temperature influence microbiological detection rate. Furthermore, the extended specimen transportation time affects the diagnosis when a low number of pathogens is present. A source of error is the presence of antibiotics in the puncture fluid if an anti-infective therapy has already been started. Therefore, an antibiotic-free interval before surgery is useful. The use of blood-culture media is also warranted to improve infection diagnosis [19]. An advantage of this modern media is that it contains charcoal or resin. Both additives are able to bind substances (e.g. antibiotics) that potentially inhibit bacterial or fungal growth.

The paediatric blood-culture bottles used in this study have the advantage of being optimised for volumes of one to three. However, they do not support the growth of anaerobes. Weiss et al. showed that the use of a paediatric blood-culture system can be a reliable and fast alternative to conventional microbiological methods [19]. Spanghel et al. claimed that removal of intraoperative tissue samples was the gold standard in the diagnosis of prosthetic infection due to the inaccuracy of Gram staining [15]. In 2005, Ochs et al. recommended native samples instead of smear tests for microbiological investigations for diagnosis of orthopaedic implant infection [20];

however, even in that study, evidence for bacteria could not be found in 20 % of cases.

The aim of our study was to discover whether PCR of the biofilm extracted by scraping the prosthetic surface is a useful diagnostic tool in detecting prosthetic joint infection. Although this is an approved method, for reasons of practicality and cost we did not use ultrasound [5, 6, 9]. This is an important consideration for hospitals that do not have the facilities for ultrasound but do perform septic orthopaedic implant removal surgery. In 2009, Bjerkan et al. found ultrasound superior to scraping in vitro [7].

Using conventional microbiological methods has its limitations, and PCR has improved diagnostic quality in recent years [8, 10, 21–24]. Only broad-range PCR fails to show better results than conventional methods, as Panousis et al. demonstrated in 2005 [25].

This study has several limitations. One is the possibility of contamination and the detection of the contaminant's bacterial DNA. By accurately processing and handling all specimens, we tried to minimise the possibility of contamination. In particular, we looked at patients with pathogens detected only by PCR of biofilm; two of them had no clinical signs of infection. As they had pain-related problems with their arthroplasty, they underwent surgical removal for aseptic loosening. The pathogens detected could be the reason for the disease: a low-grade infection. After arthroplasty removal and antibiotic treatment, the condition of the patients improved. Another limitation is that an antibiogram with PCR was not produced, as few parameters were available (e.g. the *mecA* gene for methicillin-resistance in staphylococci). However, this additional examination will be performed in a follow-up study in which the diagnostic quality of PCR and microbiological methods will be compared. PCR was able to detect additional bacteria in two cases and was the only diagnostic tool for detection in three cases. Although the sample size of 37 is small, we can tentatively state that PCR of biofilm improved the detection rate by 8.1 %.

In this study, we demonstrate that 16S rDNA PCR is a simple method and a valuable addition to the diagnostic assessment of prosthetic infection and, along with conventional microbiological methods, should be part of an ideal standard algorithm.

Conflict of interest The authors declare that they have no conflict of interest.

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