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Poor predictive value of broad-range PCR for the detection of arthroplasty infection in 92 cases

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Background The diagnosis of prosthetic infection remains a challenge, as no test is 100% sensitive and 100% specific. Recent advances in molecular biology have enabled the detection of infection in culture negative cases.

Patients and methods We evaluated the effectiveness of polymerase chain reaction (PCR) in detecting infection in failed joint replacements prospectively in 91 consecutive patients (92 prosthetic joints) undergoing revision total hip or knee arthroplasty. Synovial fluid was collected intraoperatively and examined by broad-range PCR assay for detection of bacterial DNA. The clinical diagnosis of infection was based on the results of blood tests, preoperative joint aspiration, culture and histology of multiple intraoperative tissue samples, as well as the surgeon's assessment.

12 joints (13%) were infected, but the PCR was positive in 32 cases. The sensitivity of the technique was 92%, the specificity 74%, the accuracy 76%, the positive predictive value 34%, and the negative predictive value was 98%.

Interpretation The PCR technique cannot be recommended for the routine detection of prosthetic infection. The large number of false positive results may represent sample contamination, or bacterial presence related to low-virulence organisms, low bacterial load, or a strong host immune response.

infection requires a range of investigations, including blood tests (ESR and CRP), Technetium-99m and Indium-111 leukocyte isotope scans, preoperative joint aspiration, and the culture and histological examination of intraoperative tissue samples (Glithero et al. 1993, Windsor and Bono 1994, Fitzgerald 1995, Spangehl et al. 1999, Teller et al. 2000, Joseph et al. 2001). However, as no test is 100% sensitive and 100% specific, the diagnosis of infection remains a diagnostic challenge.

The polymerase chain reaction (PCR) is a molecular biological technique that enables the production of large amounts of DNA from—at least in theory—one original DNA molecule. PCR has made possible the detection of rare DNA and RNA sequences and has been used for the diagnosis of a variety of genetic and infectious diseases (Saiki et al. 1985, Ni et al. 1992, Greisen et al. 1994, Lu et al. 2000, Rantakokko-Jalava et al. 2000). PCR has been used in the field of musculoskeletal infection (Muralidhar et al. 1994, Wilbrink et al. 1998, Jalava et al. 2001, Titov et al. 2004) and in the detection of prosthetic infection (Mariani et al. 1996, Hoeffel et al. 1999, Tunney et al. 1999).

We evaluated the use of a general PCR for the diagnosis of bacterial infection in failed hip and knee joint replacements.

Patients and methods

We assessed prospectively 91 consecutive patients (92 prosthetic joints) awaiting revision total hip or knee arthroplasty. There were 56 women and 35

Infection is the cause of failure in 1–2% of primary total hip replacements (Spangehl et al. 1999) and in 1.1–12% of primary total knee replacements (Windsor et al. 1990). The diagnosis of prosthetic

men, with a mean age of 66 (24–85) years. In 76 cases the hip joint was revised, and the remaining 16 cases were knee revisions. The indication for the initial procedure was osteoarthritis in 63 joints (54 hips and 9 knees), rheumatoid arthritis in 11 joints (6 hips and 5 knees), developmental dysplasia of the hip (DDH) in 5 hips and avascular necrosis in 5 hips. The remaining 8 joints (6 hips and 2 knees) had other diagnoses.

Preoperative assessment was based on patient history, clinical examination, blood tests and plain radiographs. An ESR of more than 30 mm/h and a CRP of more than 10 mg/L were considered to be indications of infection. Only patients with suspected infection had their joint aspirated prior to the revision operation. When the result of aspiration was negative despite strong suspicion of an infection, intraoperative frozen section was used to ascertain whether infection was present.

Revision surgery was performed in an operating theatre with laminar airflow ventilation. Antibiotics were withheld until all synovial fluid and tissue samples had been obtained. Synovial fluid was aspirated before capsulotomy, taking care to avoid contact between the needle and the instruments used. Part of the synovial fluid sample was immediately injected into a sterile vial and sent for PCR. The remaining fluid was used to inoculate two blood culture bottles containing aerobic and anaerobic culture media and charcoal to absorb growth inhibitors. The bottles were incubated in an automated detection system. At least 3 tissue samples were taken from the pseudocapsule, femoral membrane and either acetabular or tibial membrane, as well as from the most inflamed areas. These were sent for bacteriological and histological examination.

Tissue specimens were considered positive for infection if more than one-third of the cultures showed bacterial growth (Spanghehl et al. 1999). Histology was considered positive for infection if any single HPF contained at least 5 stromal neutrophils (Mirra et al. 1982), but intravascular neutrophils embedded in fibrin or necrotic tissue were excluded.

As no universally accepted definition of infection exists, the final determination of which joints were classified as infected was based on the surgeon's opinion, which encompassed the pre- and intraoperative findings and the results of radiographic and

laboratory investigations (Spanghehl et al. 1999, Gambhir et al. 2000).

Infected arthroplasties were revised according to a two-stage protocol with an interval of at least 6 weeks between prosthesis removal and reconstruction. All patients were seen at 6 weeks, 3 months and 6 months postoperatively, and annually thereafter. All patients had postoperative follow-up of at least 2 years.

PCR testing

The bacteria causing infections of prosthetic joints all possess a highly conserved gene encoding the 16s ribosomal subunit—16S rRNA (Brosius et al. 1978, Lu et al. 2000). Consequently, we used a single set of PCR primers that target the 16S rRNA gene to amplify DNA from several bacterial species (Mariani et al. 1995).

Bacterial DNA from the synovial fluid samples was extracted using a lysis and extraction buffer containing proteinase K. A mixed bed, ion exchange resin was then added and the solution was centrifuged. The supernatant was removed and used for PCR which was done according to standard procedures. The reaction was run in a thermocycler for 35 cycles. Each cycle consisted of 3 steps; a denaturation step of 50 sec at 95°C, an annealing step of 50 sec at 65°, and an extension step of 90 sec at 72°C. Finally the PCR product was size fractionated by gel electrophoresis and visualized by ethidium bromide staining under ultraviolet light.

Positive and negative extraction and reagent controls were set up to exclude contamination. As a positive control we used samples of bacterial DNA extracted from a synovial fluid specimen that was positive on bacteriological culture and strongly positive in PCR. A synovial fluid sample that showed no growth was used as a negative control. A blank-negative PCR sample was also prepared using steam sterilized, UV irradiated water. These three samples were run parallel with the test samples. In addition, a DNA sample extracted from stimulated mononuclear cells positive for β 2-microglobulin was tested each time, to verify the integrity of the PCR reaction (housekeeper gene).

To prevent contamination of the PCR assay in the laboratory, several measures were taken. DNA extraction was done in a separate area from the one used for preparation of reagents and the actual PCR.

Table 1. Pathogens isolated from preoperative aspiration

	Hips	Knees	Total
Coagulase-negative			
<i>Staphylococcus</i>	3	1	4
<i>Staphylococcus aureus</i>	3	0	3
<i>Enterococcus</i>	1	0	1
<i>Streptococcus sanguis</i>	1	0	1
Group A <i>Streptococcus</i>	1	0	1

Table 2. Bacteria isolated from intraoperative cultures from infected joints. Specimens from 2 hips grew more than 1 type of organism

	Hips	Knees	Total
Coagulase-negative			
<i>Staphylococcus</i>	5	1	6
<i>Staphylococcus aureus</i>	1	1	2
<i>Streptococcus viridans</i>	2	0	2
<i>Enterococcus</i>	2	0	2
Diphtheroids	2	0	2

Table 3. Contaminants isolated from intraoperative cultures. Specimens from 2 hips grew more than 1 type of organism

	Hips	Knees	Total
Coagulase-negative			
<i>Staphylococcus</i>	4	1	5
<i>Streptococcus viridans</i>	1	0	1
<i>Streptococcus mitis</i> (<i>viridans</i> group)	0	1	1
Group D <i>Streptococcus</i>	2	0	2
<i>Streptococcus</i>	1	0	1
<i>Candida</i>	1	0	1

The PCR reaction mixtures were prepared in a class II hood whereas fluids and DNA were handled in a separate flow hood. All glassware and spatulas used to prepare reagent solutions were heated at 250°C for at least 6 h. Water used was treated with diethylpyrocarbonate (DEPC) to ensure that it was DNase- and RNase-free. Prepacked gamma-irradiated sterile pipettes and containers were used. Plastics, pipettes and reagents were UV-irradiated in a flow hood for 30 min before use.

Results

12 (13%) of the 92 failed prosthetic joints, 10 hips and 2 knees, were diagnosed as being infected. In 57 cases the ESR was less than 30 mm/h, and in 35 cases it was more than 30 mm/h. In 55 cases the CRP was less than 10 mg/L, and in 37 cases it was more than 10 mg/L. 48 joints (40 hips and 8 knees) with suspicion of infection were aspirated. 2 joints, 1 hip and 1 knee that eventually proved to be infected, were not aspirated. The pathogens isolated from the preoperative aspiration are shown in Table 1. The histological examination of the intraoperative tissue samples was positive for infection in 11 cases (9 hips and 2 knees) and negative in 81 (67 hips and 14 knees). The intraoperative cultures yielded a variety of organisms. In 71 cases (59 hips and 12 knees), all specimens were negative. In 12 cases, the same organism was isolated from more than one-third of the specimens. The organisms cultivated are shown in Table 2. In 9 cases (7 hips and 2 knees) less than one-third of the tissue specimens were positive, thus representing contamination. The organisms that were cultured are shown in Table 3. PCR was positive in 32 cases (26 hips and 6 knees) and negative in 60 cases (50 hips and 10 knees).

The sensitivity, specificity, accuracy, positive and negative predictive values were determined for the results of ESR and CRP, of the preoperative aspiration and of the intraoperative tissue culture and histology, as well as those of the PCR. The results are summarized in Table 4. At the 2-year follow-up, there were no patients with evidence of infection or recurrence of infection in their revised joints.

Discussion

In this study, intraoperative cultures and histology were the most reliable tests for diagnosis of bacterial infection. These findings are in agreement with those of previous studies (Barrack and Harris 1993, Lonner et al. 1996, Spanghel et al. 1999). In order to minimize the false negative results of synovial fluid cultures (because of the presence of polymerase enzyme inhibitors (Mariani et al. 1995)), we used blood culture bottles (von Essen and Holta 1986). These allow the detection of very

Table 4. Results of tests used for the detection of prosthetic infection

	Sensitivity	Specificity	Accuracy	Positive predictive value	Negative predictive value
ESR	0.75	0.68	0.68	0.26	0.95
CRP	0.67	0.64	0.64	0.22	0.93
Aspiration	0.7	0.95	0.9	0.78	0.92
Tissue culture	0.75	0.96	0.93	0.75	0.96
Histology	0.92	1	0.99	1	0.99
PCR	0.92	0.74	0.76	0.34	0.98

low numbers of bacteria, and dilute out growth inhibitors. The addition of anti-antibiotics (such as β -lactamase or p-aminobenzoic acid) and absorbing factors (charcoal) may enhance the results of the method, especially when the patients are on antibiotic therapy. Intraoperative frozen sections of periprosthetic tissues can provide decisive help regarding one versus two-stage revision in cases with contradictory findings (Athanasou et al. 1995).

The false positive PCR results may be due to contamination in the operating theatre by skin flora, or by DNA from nonviable cells present in the equipment and vials used for the collection of the sample (Keay et al. 1998). Contamination can also occur in the laboratory from the skin flora of the personnel, the PCR reagents, especially the DNA polymerase enzyme and the equipment used in the PCR reaction (Carroll et al. 1999, Corless et al. 2000, Millar et al. 2002). Strict segregation of the laboratory workflow has been proposed as a fundamental requirement for successful broad-range PCR (Millar et al. 2002). Implementation of this requirement however, renders PCR very expensive and possibly prohibitive for small laboratories.

Another explanation for PCR-positive results in apparently noninfected cases could be the detection of a bacterial load in the wound that fails to express clinical infection. This could be due to the low virulence of the organisms, the low number of bacteria, or a strong host immune response that controls infection (Mariani et al. 1996). Davis et al. (1999) found 63% contamination of the surgical equipment used in 100 primary arthroplasties, but after two years only 1% of patients had developed infection. Bacterial fragments or nonviable bac-

terial DNA debris that remain in the tissues after antibiotic treatment for established infection may give rise to a positive PCR result (Ni et al. 1992, Canvin et al. 1997). The use of preoperative antibiotics could have similar effects. Bacterial DNA has been detected by PCR in blood samples from healthy volunteers (Nikkari et al. 2001) and in synovial fluid samples from patients with inflammatory arthritis (Wilbrink et al. 1998, Wilkinson et al. 1999). False negative PCR results can be attributed to the presence of polymerase enzyme inhibitors contained in the synovial fluid (Mariani et al. 1995), failure to extract bacterial DNA because of the thick cell wall of Gram-positive cocci, or the loss of bacterial DNA during purification (Trampuz et al. 2003).

Mariani et al. (1996) studied 50 failed TKRs using PCR on synovial fluid and concluded that the method provides an additional assay for identification of prosthetic infection. Tunney et al. (1999) examined sonicates from 120 retrieved hip prostheses using culture, immunofluorescence microscopy and PCR. They detected bacterial DNA in 72% of specimens and concluded that the incidence of prosthetic joint infection is grossly underestimated by current culture detection methods. Hoeffel et al. (1999) evaluated 69 patients undergoing revision THR and found that PCR had a sensitivity of 71% and a specificity of 49%.

PCR assay with universal primers cannot specify the bacteria implicated in a prosthetic infection. Further multiplex PCR with species-specific primers is required for species identification. To overcome these problems, reverse transcription-PCR has been introduced. This technique targets the 16s-rRNA, thus proving the existence of transcriptionally active bacteria, and has increased sen-

sitivity compared to r-DNA PCR. Bacterial RNA is extracted and reverse transcriptase is used to synthesize complementary r-DNA. The PCR product is subsequently cloned into a cloning vector and sequenced, thus allowing identification of specific bacteria (Kempell et al. 2000). Real-time quantitative PCR is a refined technique that avoids contamination of the assay by using a closed-tube system and is more sensitive than traditional PCR (Schmittgen 2001). It offers the possibility of running simultaneous multiplex reactions and avoids post-PCR manipulations.

In our study, broad-range PCR was not helpful as a screening test for the identification of prosthetic infections. The clinical relevance of such PCR-positive results remains unclear. Even so, we have found the use of conventional methods of culture and histology combined with the surgeon's clinical impression to be of sufficient accuracy in detecting the presence of prosthetic joint infection.

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