Detection of anaerobic prosthetic joint infection by PCR and DNA sequencing—a case report

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A 66-year-old woman fell off her bicycle and acquired a comminuted fracture and dislocation of the left patella 6 months after she had undergone total knee replacement (TKR), including a patella surface prosthesis due to severe arthrosis of the left knee. Until the accident, the rehabilitation had progressed normally.

Osteosynthesis of the patella was performed, leaving the patella implant in situ. The patient was given 1.5 g cefuroxime preoperatively. One week later, the patella again dislocated because of rupture of the osteosynthesis without any signs of infection, and another operation was performed.

Part of the exposed patella was removed and the remaining part was fixed to the inferior ligament of the patella. Cefuroxime was administered preoperatively and also 3 days postoperatively, at a dose of 1.5 g 3 times a day. Swabs taken from the joint during operation were negative by culture. 2 weeks later, serosanguinous discharge from the wound was observed. The joint was red and sore, and a C-reactive protein concentration of 198 mg/L (normal: < 5 mg/L) and blood leukocyte count of 7.0 (3.0–11.0) × 10^{10}/L were measured. The patient was afebrile. Cultures from joint aspirate showed coagulase-negative staphylococci that were resistant to dicloxacillin without growth of anaerobic bacteria after two days of incubation.

The patient was re-admitted and given Cefuroxime at a dose of 1.5 g 3 times a day. There was still serosanguinous discharge after 2 additional weeks, and plasma levels of C-reactive protein and blood leukocyte count were 58 mg/mL and 5.5 ×10^9/L, respectively. Even so, the patient could walk without any pain. However, C-reactive protein values showed persistently elevated levels (> 55 mg/mL) for 1 month despite continuous antibiotic treatment intravenously. At a third operation, 6 weeks after the arthroplasty, the patella was removed and all infected synovial tissue was removed. Septocoll plates were inserted. 5 biopsies were collected and coagulase-negative staphylococci were cultured from four of them. The pattern of antibiotic resistance was the same as for the coagulase-negative staphylococci cultured from the joint fluid aspirated 1 month earlier.

After 6 months, a fourth operation was inevitable as the biochemical markers were continuously elevated (C-reactive protein at 42 mg/L and a blood leukocyte count of 8.8 × 10^9/L). All implants were removed and an arthrodesis with ring fixation was performed. During the operation, 5 samples of synovial tissue were collected and coagulase-negative staphyloccoci were cultured from one. The strain was not identical to the strain cultured in the previous samples, as the antibiotic resistance profiles differed. In addition, anaerobic cocci characterized as *Peptostreptococcus sp.* were cultured from 2 samples.

After the removal of all foreign body material, the patient was treated for another 10 weeks with dicloxacillin (1 g 4 times a day) until the blood samples showed normal values of C-reactive protein. Finally, 10 months after the accident, the patient had the ring fixation apparatus removed; the arthrodesis was healed.

4 of the original tissue samples from the revision were analyzed retrospectively by PCR amplification of a 526-base pair (bp) stretch of the 16S rRNA gene of chlorogloeposis HTF strain PCC7518 (Wilmotte et al. 1993) and subsequent DNA sequencing of the PCR product as described previously (Kemp et al. 2005). The DNA sequences obtained were compared to sequences deposited in the NCBI database using the BLAST search engine.

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DNA from *Finegoldia magna* was detected in 3 of the 4 samples with 436/443 (98%), 341/353 (96%), and 158/178 (88%) identical base pairs, respectively. Second best taxon match of the DNA sequences from the same 3 samples were: *Peptostretpcoccus micros* with 314/354 (88%) identical base pairs, *Peptostreptococcus micros* with 226/246 (91%) identical base pairs, and *Anarococcus vaginalis* with 143/168 (85%) identical base pairs. Culture from 1 of these samples had been negative for bacteria, while *coagulase-negative staphylococci* had been isolated from the other 2.

Discussion

Culture-independent amplification of bacterial DNA and subsequent identification of infecting bacteria by DNA sequencing is increasingly used in the diagnosis of culture-negative infections. While exploiting the usefulness of the method, we encountered a case of anaerobic prosthetic knee infection, which had not been detected by culture. Despite the growth of coagulase-negative staphylococci only, a subsequent PCR analysis for bacterial DNA and subsequent sequencing of the amplified DNA showed the presence of DNA from the strictly anaerobic coccus *Finegoldia magna* in the joint. 6 months after the operation, the prosthetic joint was removed—and at that time the anaerobic cocci were cultured. Although these bacteria had not been finally identified, they were most probably the same as those demonstrated by molecular techniques in samples from the first operation.

Finegoldia magna, previously called *Peptostreptococcus magnus* is one of the Gram-positive anaerobic cocci most commonly isolated from human clinical specimens (Murdoch 1998). The bacterium can cause chronic, low-grade but painful posttraumatic and postoperative infections, which usually necessitate aggressive surgery and prolonged treatment with antibiotics for successful eradiation. The major sites of inhabitation are the skin, oropharynx, upper respiratory tract, gut, and urogenital tract (Murdoch 1998). *F. magna* has been isolated from prosthetic knee joint infections previously, and identified by culture-dependent methods (Davies et al. 1988).

Molecular methods are more accurate in identification of Gram-positive anaerobic cocci than phenotypic characterization (Song et al. 2003). Moreover, molecular methods are being increasingly used for identification of pathogenic bacteria that cannot grow on standard media—or where antibiotics have been administered before samples are obtained (van der Heijden et al. 1999). In such cases, culture-independent DNA amplification of the 16S rRNA gene and subsequent species identification by DNA sequencing may prove useful.

There may be several reasons for unsuccessful culture other than those mentioned above. Moisture is an important factor for maintaining viability during transport and storage of the specimens (Brook 1987), and anaerobic bacteria are susceptible to exposure to oxygen before being processed in the laboratory. Furthermore, *F. magna,* which was positively identified by partial 16S rRNA gene amplification and DNA sequencing in this report, rapidly loses viability when exposed to oxygen (Tally et al. 1975).

The incidence of prosthetic infections is probably highly underestimated when culture is used as the only bacterial detection method (Tunney et al. 1999). It has been suggested that formation of biofilm on the surface of the foreign body material may result in viable—but non-cultivable—bacteria, which can further contribute to this underestimation (Tunney et al. 1999). In our case, PCR and DNA sequencing resulted in demonstration of *F. magna* despite the growth of coagulase-negative staphylococci. Microscopy of the specimens showed Gram-positive cocci occurring in clusters, which would be the expected appearance of both coagulase-negative staphylococci and *F. magna.* It is plausible that both bacteria were present, but in different quantities, and that the non-linear PCR amplification resulted in generation of detectable amounts of PCR product from *F. magna.* DNA from *coagulase-negative staphylococci* was possibly present in such small quantities that it remained undetected by PCR and DNA sequencing, and consequently did not interfere with the sequencing of *F. magna* PCR amplicons. The primers used are homologous to most bacteria.

It is possible to separate PCR products from different species of bacteria in the same sample by denaturating gradients (Davies et al. 2004), and

subsequently identify the individual bacteria by DNA sequencing. By using such techniques in the future, it will be possible to identify all bacteria in multi-bacterial infections by molecular methods. This is particularly important in foreign-body infections and other orthopedic infections, which are often multi-bacterial in nature. Thus, routine application of procedures for separating amplicons will allow full exploitation of the molecular diagnostic techniques without the risk of overlooking bacteria that are present in small quantities.

Speed is a crucial factor for the value of any routine diagnostic procedure. With an appropriate setup in the laboratory, final bacterial diagnosis can be obtained within a few days. Routine implementation of faster techniques such as real-time PCR and pyrosequencing will increase the speed further and improve the clinical relevance of molecular diagnostics. In their present form, however, these methods have limitations which must be overcome. For example, pyrosequencing is only possible for short DNA amplicons.

Anaerobic bacteria are probably underestimated as agents of orthopedic foreign body infections. By presenting this case, in which anaerobic bacteria were detectable by PCR and DNA sequencing in samples 6 months before the bacteria were detected by culture, we are drawing attention to the problem and to the usefulness of PCR and DNA sequencing in detection of such infections.

Contributions of authors

HH and NS: primary responsible for clinical data collection. KA, JJC, MK: responsible for laboratory work and interpretation. All authors contributed to writing of this report.

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