Case Report

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Genotypic versus phenotypic methods in the detection of *Listeria monocytogenes* prosthetic joint infection

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A rare case of a severe prosthetic joint infection in a 71-year-old immunocompetent woman is presented. *Listeria monocytogenes* was identified in two consecutive samples using broad-range PCR and sequencing, whereas cultivation remained negative for the first sample and streptococci of a non-group A streptococci, non-group B streptococci type were detected for the second one. This report demonstrates that the phenotypic approach may lead to misidentification of *L. monocytogenes* in a routine clinical setting. Molecular methods of pathogen detection might be useful when a rare and/or unexpected micro-organism is present or the sample is collected during antibiotic treatment.

Received 29 August 2008
Accepted 9 February 2009

Introduction

In recent years, prosthetic joint replacement has shown great success in restoring the function of joints in persons disabled by arthritis or osteoarthritis. However, in a subgroup of patients, implantation of a foreign body into the joint carries a high risk of bacterial infection that may result in serious complications and even in the patient's death (Zimmerli, 2006). Fast and accurate microbiological diagnostics are needed to allow detection of the infectious agent and initiation of targeted antibiotic therapy as early as possible.

Clinical tests based upon microbiological phenotypic features are considered the diagnostic standard for detection and identification of causative pathogens in many countries, including the Czech Republic. However, considerable limitations of cultivation-based tests, such as the length of time to obtain a result, misidentification of species due to aberrant phenotype and false-negative results in cases of fastidious micro-organisms or ongoing antibiotic treatment, have been described by a number of authors (Petti *et al.*, 2005; Rantakokko-Jalava *et al.*, 2000). Genotypic methods based on 16S rRNA gene sequencing have become useful tools for recovering the infectious agent in culture-negative bone and/or joint infection cases (Fenollar *et al.*, 2006; Gallo *et al.*, 2006; Wilkinson *et al.*, 1999; Drancourt *et al.*, 2000). Here we present a case of a

71-year-old woman with two episodes of a prosthetic joint infection (PJI), and discuss the conflicting results between routine cultivation and 16S rRNA gene sequencing in the identification of the causative pathogen.

Case report

A 71-year-old woman with prosthetic knee joints came to the hospital with progressive pain in her left knee. The patient's past medical history included heavy gonarthrosis in both of her knee joints. She had undergone a total left and right knee replacement 7 and 2 years ago, respectively. Except for chronic bronchitis, treated hypertension and hypothyroidism, she had no signs of any underlying disease.

Fifteen days prior to hospital admission, she was treated for fever and pain in her left knee by her general practitioner. However, after a 2 week combined oral amoxicillin–clavulanate therapy ended, her problems reoccurred. She was admitted to the Trauma Hospital of Brno with suspected bacterial infection of the prosthesis and the adjacent joint. Laboratory results showed signs of infection, with C-reactive protein levels of 221 mg l⁻¹. Surgical revision, including synovectomy, debridement and gentamicin lavage, was performed under intravenous amoxicillin–clavulanate coverage (1.2 g three times a day). The prosthesis was retained. Histological examination showed a lamellar structure of necrotic granulation, as well as fibrous tissues with numerous polymorphonuclear leukocytes in

Abbreviations: GAS, group A streptococci; GBS, group B streptococci; PJI, prosthetic joint infection.

the synovium. Synovial fluid, but no tissue sample, was available for microbiology testing by both standard culture techniques in a local microbiology laboratory (Department of Clinical Microbiology, St Anne's University Hospital, Brno, Czech Republic) and by molecular methods using broad-range PCR in our laboratory (Molecular Genetics Laboratory, Centre for Cardiovascular Surgery and Transplantation).

A set of non-selective and selective planting media routinely used for synovial fluid samples was applied to detect the causative pathogen. If positive in brain heart infusion broth (Difco) (the second sample collected 4 months later), the suspension was planted onto non-selective Endo agar (Imuna) and standard sheep blood agar (Oxoid), which was further incubated under both aerobic and anaerobic conditions. A selective sheep blood agar with 10% NaCl was used for specific detection of possible staphylococci, whereas possible streptococci were specifically detected on a selective sheep blood agar with amikacin. For molecular examination microbial DNA was isolated from a synovial fluid sample using a DNA blood mini kit (Qiagen). PCR was performed with universal eubacterial primers targeting V8 and V9 regions of the 16S rRNA gene. The PCR mix was decontaminated with 8-methoxypsoralen and UV light cross-linking before template DNA was added. A direct sequencing procedure was carried out using an ABI Prism 3100 Avant (Applied Biosystems) followed by sequence alignment analysis with the GenBank database (<http://www.ncbi.nlm.nih.gov/blast>).

Cultures remained negative, while the molecular approach led to the identification of *Listeria monocytogenes* (100% sequence homology to the sequence with GenBank accession no. AL591981). The patient was given oral penicillin V (0.5 g four times a day) for the next 6 weeks. She was discharged in a stable condition 12 days after surgery.

Four months later, the pain and fever (39.1 °C) reoccurred. The joint was oedematous, with X-ray films demonstrating the tibial component of the prosthesis becoming loose. Preoperative synovial aspirate showed leukocytes by microscopy, and β -haemolytic streptococci after 48 h of aerobic cultivation on standard blood agar. Both the PYR (pyrolidonyl acrylamidase) and CAMP (Christie, Atkins, Munch, Petersen) reaction for detection of group A streptococci (GAS) and group B streptococci (GBS), respectively, gave negative results. The strain was susceptible to penicillin, erythromycin, chloramphenicol, vancomycin and doxycycline, and resistant to cefalotin and clindamycin. Molecular examination of this sample in our laboratory revealed the same strain of *L. monocytogenes* as found earlier.

Four days prior to surgical intervention, the patient was given oral penicillin V (0.5 g four times a day). Implant removal, debridement, necrectomy, gentamicin lavage and implantation of a cement spacer with gentamicin were

performed. Six days after surgery, the patient was given intravenous penicillin G (5×10^6 units four times a day) and gentamicin (240 mg). Subsequently, G-penicillin was replaced with oral penicillin V (0.5 g four times a day). Both standard bacteriological examination and PCR testing of synovial fluid gave negative results 1 day after surgery. One year after surgery, the knee showed no signs of infection and a new prosthesis was implanted.

Discussion

In modern orthopaedics, hip and knee replacements are among the most common implants. Although the incidence of prosthetic knee joint infection is about 2–5%, these infections remain a diagnostic and therapeutic problem. An increase in the number of infectious complications in the last few years has been mainly boosted by the growing size of the elderly population, who are most likely to require implantation (Anguita-Alonso *et al.*, 2005).

In this study, both molecular and culture methods were used to identify the pathogenic agent in the elderly patient with PJI. Using a molecular approach, *L. monocytogenes* was identified in two consecutive synovial fluid samples, while both aerobic and anaerobic culture of the first sample remained negative, and β -haemolytic streptococci of non-GAS, non-GBS type were reported after 48 h cultivation from the second sample collected 4 months later.

Since a great susceptibility of the broad-range PCR system to contamination with exogenous DNA has been reported elsewhere (Corless *et al.*, 2000), we routinely used controls for possible contamination and potential PCR inhibition during DNA isolation and PCR mix preparation. Our assumption that *L. monocytogenes* is the causal pathogen rather than a contaminant is supported by the detection of the same sequence showing 100% homology in both independent samples. Moreover, we have never observed contamination of our clinical samples by *Listeria* spp. and both negative controls showed no signal. During the last 4 years we have examined more than 400 joint and/or bone samples with suspected infection, and detected *L. monocytogenes* in only three cases at different times (two of them reported here), which makes the presence of a laboratory contaminant in our samples even more unlikely.

We suspect that the first sample was culture-negative due to antibiotics given during the time of material collection, and that the pathogen was not completely eliminated after the first surgery because of insufficient dosage and duration of antibiotic therapy. It is highly probable that the second synovial fluid sample might have been misidentified by routine microbiological examination. Using blood agar and incubation conditions (35 °C for 24–48 h), *L. monocytogenes* grows from clinical specimens obtained from normally sterile sites in small grey colonies with a narrow zone of β -haemolysis (Bille *et al.*, 2003; Schuchat *et al.*, 1991). However, unlike streptococci, *Listeria* is rarely seen in synovial fluid and therefore was not expected in this type

of clinical specimen. Another possible explanation of the discrepant results between standard cultivation and molecular detection could be that two different pathogens (streptococci of non-GAS, non-GBS type and *Listeria* spp.) were simultaneously present in the material (Fenollar *et al.*, 2006). However, this was probably not the case since the sequencing electropherogram did not show a polymicrobial pattern. Unfortunately, due to a lack of communication the result of 16S rRNA sequencing from the first sample was not known to the microbiologists in the local microbiology laboratory; therefore, the sample was neither specifically examined for *Listeria* characteristics using selective media, nor specifically examined for colony growth on sheep blood agar.

Listeria joint infections are very rare events; a Medline search revealed only 35 reported cases, with about two-thirds of them occurring in prosthetic joints (Marculescu *et al.*, 2006; Schett *et al.*, 2005; Gómez-Rodríguez *et al.*, 2006; Kesteman *et al.*, 2007; Cone *et al.*, 2008; Tien *et al.*, 2008; Handrick *et al.*, 2008). In a recently published series of 525 samples from patients with suspected bone and/or joint infection, no *L. monocytogenes* was detected by either standard or molecular methods (Fenollar *et al.*, 2006).

Our patient was elderly with a prosthetic joint, but had no underlying disease and did not receive any immunosuppressive drugs, in contrast to the majority of described cases. She atypically developed late PJI (7 years after surgery), probably as a result of transient bacteraemia due to the ingestion of contaminated food. The initial treatment included thorough debridement, and a combined β -lactam and aminoglycoside therapy. However, severe infectious joint complications appeared again 4 months later, possibly due to an insufficient dosage and duration of the antibiotics given. Due to a large bone defect, a two-stage surgical approach was finally deemed necessary.

It has been shown here that molecular methods based on 16S rRNA sequencing provide a useful adjunct to conventional methods, and may help prevent laboratory errors in the detection of unusual pathogens. This case highlights the need for tight collaboration between microbiologists, molecular microbiologists and orthopaedic surgeons to successfully treat patients with recurrent prosthetic joint infections.

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