Case Report Article

Application of a sonication fluid vial culture method to diagnosis of prosthetic knee joint infection caused by Granulicatella adiacens

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Abstract

Prosthetic joint infection is a rare but severe complication of arthroplasties, quite challenging to diagnose, especially when the pathogenic microorganisms are difficult to isolate. Granulicatella, a nutritionally deficient microorganism, is a normal component of the oral flora which under specific circumstances may be pathogenic. We report a prosthetic knee joint infection by Granulicatella adiacens, 8 years after total arthroplasty, on a 76-year-old woman. Laboratory diagnosis was achieved via a novel combined technique, using the pioneering sonication method on the implant and inoculation of the sonication fluid in a pediatric blood culture bottle (sonication fluid vial culture). This technique requires further investigation since its promising results appear to open a new direction in diagnosis of prosthetic joint infections.

Keywords: Sonication fluid vial culture, Granulicatella, Prosthetic joint infection, Nutritionally variant streptococci, Arthroplasty

Introduction

Orthopedic-implant-associated infection is a relatively rare complication of primary arthroplasties (1-2%). In the recent years, however, an increase in such infections is observed as the joint replacement prosthesis has become a common procedure in orthopedic surgery1. Orthopedic implant-associated infections pathogenesis is related to the microorganism ability to grow in biofilms, embedded in a polymeric matrix. The biofilm protects microorganisms from antibiotics as well as the host’s immunity mechanisms. These microbes are difficult to separate from the biofilm, which renders diagnosis of such infections particularly difficult2. Therefore, we presume that low infection rates reported tend to be underestimated, as low-grade infection is often perceived as aseptic failure. An important tool for diagnosis of such infections is a novel method, namely sonication of the removed implants, followed by culture of sonication fluid, first described and standardized by A. Trampuz a few years ago3. The conventional culture methods applied till then, using synovial fluid or intraoperative tissue for culturing, are characterized by low sensitivity (e.g. about 54% using intraoperative tissue), with 10-30% false negative results, and/or lack of specificity4. Sonication of explanted implants may attain removal of the attached biofilm. The fluid cultured after sonication is enriched in biomembrane bacteria, thus increasing sensitivity of the method (about 75%). The sonication method has been applied to orthopedic implants, breast implants, neurosurgical shunts and cardiac devices5-8. Granulicatella belongs to the streptococcus family, more specifically the “nutritionally variant streptococci”. It was described circa 1960 by Frenkel and Hirsch9 in an endocarditis case. In 2000 it has been classified as a separate genus and subdivided into 3 different species, namely G. adiacens, G. elegans and G. balaenopterae10. G. adiacens is a catalase-negative and oxydase-negative.

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facultatively anaerobic, Gram-positive coccus and belongs to the Carnobacteriaceae\textsuperscript{11}. It is a nutritionally deficient coccus since it requires presence of pyridoxal and other additional agents for growth, it grows slowly in culture and exhibits satellitism, i.e. better growth around other bacteria colonies\textsuperscript{9,12}. Due to these features, isolation of \textit{G. adiacens} is difficult. It is a normal component of the oral, urogenital and intestinal flora\textsuperscript{13}. In rare cases it may be pathogenic, causing endocarditis, meningitis and osteoarticular infections\textsuperscript{14-19}. Currently, only 3 cases of periprosthetic joint infections caused by \textit{Granulicatella spp} have been described\textsuperscript{20-22}.

**Case report**

A 76-year-old woman admitted in our hospital with intense pain on left knee having great difficulty to walk independently. Eight years ago, the patient had a primary total knee arthroplasty due to severe osteoarthritis. Her postoperative clinical course was satisfactory, with good joint functionality. Conventional X-rays showed loosening of the primary knee replacement with subsidence of the tibial component (Figure 1). The main laboratory findings upon arrival were: CRP < 0.345 mg/L (normal range <0.3), ESR= 80 mm/h (normal range 0-20 mm/h ) and WBC= 6.45×10\textsuperscript{3}/Ml (normal range 4-10/Ml). She was subjected to three-dimensional tomography of the left knee. The tomography showed a medium fluid concentration in the joint behind the patella, bone resorption at the tibial side along with some bone loss below the migrated tibial component, mild forward subluxation of the femoral component with respect to the tibia and some signs of degeneration of the patellar cartilage (non-resurfaced patella in the initial knee arthroplasty). An operation was scheduled 10 days later for revision arthroplasty. Two doses of teicoplanine were administered as preoperatively chemioprophylaxis (800 mg X2) on the day of surgery according to the institutional protocol. During the operation, a significant amount of pus and bone destruction was observed (Figure 2). The infected prosthetic joint was removed, extensive surgical cleaning was carried out, and 6 samples of periprosthetic tissue along with the prosthetic joint were sent to the laboratory for cultivation. A temporary antibiotic-loaded cemented prosthesis (spacer) was applied. The sonication method was applied to the prosthetic materials culture. The sonication fluid was cultivated in Columbia agar under aerobic, microaerophilic and anaerobic conditions for 15 days. Further, 4 ml of sonication fluid were inoculated in a pediatric blood culture bottle of the Bac-T Alert system, as well as in thioglycolate broth to check for possible contamination. The tissue samples, after 24 hours in nutrient broth, were cultivated on a Columbia agar plate, under aerobic and anaerobic conditions. Gram stain of the sonication fluid was negative. Culture results of the sonication fluid, the 6 tissue samples and the thioglycolate broth were all negative. On the 3\textsuperscript{rd} day after inoculation of sonication fluid in the blood culture bottle, the bottle was flagged positive. The bottle fluid was cultivated on chocolate and blood agar plates, where alpha hemolytic colonies were observed. Gram stain showed Gram-positive cocci in chains. Using the VITEK 2 system, \textit{Granulicatella adiacens} was identified. Confirmation followed by use of biochemical tests, such as API System, and satellitism test of the microbe around \textit{S.aureus}.
colonies. Antibiotics susceptibility testing was carried out by disk diffusion method. The strain was found sensitive to erythromycin, vancomycin, teicoplanin and rifampicin, and resistant to clindamycin. The patient was treated with ciprofloxacin and rifampicin. After the first stage of revision arthroplasty and antibiotic treatment, her clinical course was excellent. The second stage of revision was scheduled after 6 months. 1 ½ years after the first surgery the patient is still under monitoring and remains clinically well.

**Discussion**

Diagnosis of periprosthetic joint infections is particularly difficult, since there are no highly specific clinical or laboratory findings. Nevertheless, some generally accepted criteria, relied upon by most researchers, have been established. Thus, we consider a prosthetic-joint infection when at least one of the following criteria is met: i) visible appearance of purulence around the prosthesis region ii) sinus tract in contact with the prosthesis, iii) histopathological findings of acute inflammation, iv) increase of leucocytes with predominance of polymorphonuclear in the synovial fluid, v) positive culture from synovial fluid or periprosthetic tissue or sonication fluid. Especially regarding low virulence microorganisms, delayed and late infections are rarer than early and delayed, though they lately exhibit an increase, more pronounced in knee than in hip arthroplasties. In contrast to early infections, characterized by acute and intense onset and caused by virulence microorganisms, delayed and late infections are low grade infections caused by less virulent microorganisms. Symptoms in such cases are usually mild, such as implant loosening and persistent pain in the joint. Frequent absence of inflammation signs complicates diagnosis, so that it is often difficult to differentially diagnose from aseptic failure. In contrast to early and delayed infections where the origin is surgery related, in late infections it is haematogenous. The most often causes of bacteraemia are skin, respiratory system, dental infections and urinary tract infections. Another feature of low grade infections is the possible absence of pathological values of main infection indicators like CRP and PCT. Even in cases where increase of these indicators is observed, this usually takes place up to 2 weeks after surgery, thus being of little use to timely diagnosis of infection. In our case, the patient came 8 years after surgery, with symptoms of pain and a loose joint but no apparent clinical signs of infection since there was no fever, redness or edema in the knee area, while the only pathological laboratory finding was increased ESR. All these data along with negative cultures as well as significant pus concentration observed at surgery imply definitely a late and low grade implant-infection.

Microbiologic diagnosis was further complicated due to the negative cultures of the tissue specimens even upon application of the more sensitive and specific sonication method than the respective tissue cultures. Observation of negative cultures in implant-associated infections may be due to a variety of reasons, including antimicrobial treatment before surgery, low microbial load, prolonged time of transport to the laboratory, inappropriate culture media and fastidious bacteria. Interestingly, in our case the only positive culture was the sonication fluid inoculated into the blood bottle. *Granulicatella adiacens* is a nutritionally deficient microorganism which requires for growth mainly pyridoxal and L-cysteine, ingredients not present in the usual culture media. The pediatric blood culture bottle of BacT/Alert (Biomerieux, Marcy L’ Etoil, France) included these ingredients and hence the microorganism survived and multiplied. Its growth around a *S.aureus* colony in blood agar confirmed the identification by the automated system VITEK 2 (Biomerieux, Marcy L’ Etoil, France), since this effect, known as satellitism, is peculiar to this nutritionally deficient and fastidious microorganism. *S.aureus*, haemolyzing blood cells in Columbia agar, helps releasing pyridoxal, which is required for growth of *Granulicatella*. The identification was further confirmed via broad-range Polymerase Chain Reaction (PCR and sequencing of 16S rRNA gene). This molecular technique is, additionally, very sensitive and hence particularly useful in cases of negative findings for microorganisms of high difficulty in culturing, like *Mycobacterium spp.*, *Mycoplasma spp.*, *Granulicatella* as in our case etc., as well as in cases of antimicrobial treatment before surgery.

Treatment of the specific case followed the strategy of, revision arthroplasty in two stages, which is the method of choice in cases of unstable implant and significant damage of soft tissues. The antibiotic treatment included oral reception of rifampicin and ciprofloxacin. Rifampicin has excellent action on slowly growing microorganisms attached to the biomembrane surface, but it should never be administered as monotherapy, since it soon develops resistance. It may be combined efficiently with quinolones, especially ciprofloxacin and ofloxacin, since they have been extensively tested in studies on patients with joint infections.

Timely administering of appropriate treatment is very important in prosthetic joint infections, contributing to improvement in the patient’s quality of life as well as decrease in mortality rate. Thus, isolation of the pathogenic microorganism constitutes an important goal for clinicians and a serious challenge for laboratory physicians. To this end, various techniques have been proposed, from the conventional tissue specimen culturing method to the recent pioneering sonication method. In the present case, a novel technique has been applied, combining sonication of prosthetic materials and inoculation of the sonication fluid
in a pediatric blood culture bottle. To our knowledge, this is the first instance of isolation of *Granulicatella* in a prosthetic joint infection by the combined approach described here. The case is illustrative, since without inoculation in the blood culture bottle, isolation of the pathogen would be impossible leading to a negative result.

**Conclusion**

A spectacular increase in the number of total arthroplasties has taken place in the last few years. Since prosthetic joint infections are accordingly expected to occur relatively often, a compelling motivation arises for improvement in timely diagnosis, in pursuit of decrease in patients’ mortality rate, improvement in their quality of life and cost reduction. The pioneering sonication method, standardized by A. Trampuz and colleagues, has already contributed much in this field, while combination with other techniques such as inoculation of the sonication fluid in a blood culture bottle appears to offer additional advantages, especially in cases of nutritionally deficient bacteria. Investigation into further applications of the sonication technique in diagnosis of implant-associated infections, as well as conditions of application and possible adaptations is of strong interest for future study.

**References**

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