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ORIGINAL INVESTIGATION

Orthopedic infections caused by obligatory anaerobic Gram-negative rods: report of two cases

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Abstract Anaerobic bone and joint infections are uncommon, although the number of anaerobic infections is presumably underestimated because of difficulties with isolation and identification of obligate anaerobes. This study describes two cases of complicated *Bacteroides fragilis* peri-implant infection of the lumbar spine, infection of the hip and osteomyelitis. Bacteria were identified with the use of a mass spectrometer, VITEK MS system. Drug susceptibility was performed with the use of E-test. The EUCAST breakpoints were used for interpretation with *B. fragilis* ATCC 25285 as a control. In the two described cases clinical samples were collected for microbiological examination intraoperatively and simultaneously empirical treatment was applied. *B. fragilis* was isolated in monoculture or in a combination with other bacteria. The treatment was continued according to the susceptibility tests. In a case one clindamycin failure was observed and clindamycin resistance of the isolate was likely due to inadequate time of therapy. Difficulties in collecting an adequate samples and culturing anaerobic bacteria cause that not all infections are properly recognized. In a successful therapy, identification and determination of the susceptibility of the pathogen are essential as well as an appropriate surgical debridement.

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Introduction

Anaerobic joints, bones, bone marrow infections are uncommon. Most of the reported cases concerned post-operative infections caused by *Propionibacterium acnes* which has an ability to biofilm production. Other anaerobic bacteria, such *Clostridium* spp., *Finegoldia magna*, *Fusobacterium* spp., *Prevotella* spp., *Parvimonas* spp., *Peptostreptococcus* spp., *Peptoniphilus* spp., can be related with septic arthritis, osteitis, spondylodiscitis, and orthopedic prosthesis infections as well. Oral bacteria, for instance: *Prevotella*, *Fusobacterium*, *Porphyromonas* are most often responsible for infections within the skull, and also cause complications after bites by humans and animals [1–3].

Bacteroides fragilis is the etiological agent of infections on the hands and feet (especially in patients with vascular disease and neuropathy). It can be an etiological agent of septic infection of the large joints (e.g., hip, shoulder and knee) most commonly as a result of haematogenous spread from a distant site of infection [2, 4]. It is worth emphasizing that microbiological testing poses a number of challenges, including pre-laboratory procedures (e.g., sample selection, appropriate collection), as well as storage and transport conditions (low aerotolerance), and selection and availability to the proper laboratory diagnostic methods. Another difficulty is posed by a long-lasting cultivation. For this reason the identification with susceptibility test takes about a week. C-reactive protein (CRP) rate, erythrocyte sedimentation rate (ESR), white blood cells count (WBC) and diagnostic imaging methods are often applied

to confirm the diagnosis of infection in orthopedic patients. *B. fragilis* infections tend to be monomicrobial [1, 2, 4].

The paper describes two cases of compound *B. fragilis* orthopedic implant related infection of the lumbar spine as well as the infection of the hip and osteomyelitis. A proper laboratory diagnosis and treatment on the basis of susceptibility testing, as well as surgical debridement have caused the gradual improvement of laboratory parameters, lack of clinical signs of anaerobic infection and the decrease of pain.

Case 1

A 62-year-old man has been admitted to the orthopedic department because of a suspected implant related lumbar spine infection. The transpedicular stabilization of the lumbar spine with screws had been performed 4 months earlier due to multilevel lumbar discopathy with L4-L5 stenosis and paresis of lower limbs. Due to spinal stenosis causing paresis he has ended up being confined to a wheelchair. Three years before admission, the patient underwent a liver transplantation. His other comorbidities have included chronic insufficiency of the transplanted liver due to recurrent hepatitis C, hepatitis B infection, diabetes mellitus, and a history of invasive candidiasis treated with voriconazole. One year before he had a *Pseudomonas aeruginosa* infection of the hip confirmed microbiologically. Infection was treated with resection arthroplasty of the native joint as well as the local and parenteral antibiotics. Upon currently discussed admission the patient was afebrile; a sinus with a purulent discharge within a postoperative scar in the lumbar region was present. Inflammatory parameters were as follows: CRP 12.6 mg/L, ESR 70 mm/h, WBC 4960/μL and neutrophiles 52.4%. During the debridement operation samples from tissues adjacent to the implant and from the hip were collected, transferred to a sterile container and immediately sent to the microbiological laboratory at the room temperature. Empiric antibiotic therapy was implemented with local application of collagen-gentamicin sponge containing 130 mg gentamicin (Garamycin Schwamm, Eusa Pharma) with addition of vancomycin and cefepime and subsequent parenteral therapy with *i.v.* cefepime 1 g tid. and *i.v*. vancomycin 1 g bid. The results of microbiologic tests (identification of the bacterial species and determination of antibiotic susceptibility) were ready after 5 days. All samples were cultured on the microbiological media (Columbia agar +5% sheep blood, MacConkey agar, Sabouraud agar; all from bioMérieux, France) in the aerobic and (Schaedler agar +5% sheep blood; bioMérieux, France) on anaerobic conditions.

Bacteria were identified with the use of a mass spectrometer, VITEK MS system (bioMérieux, France). Drug susceptibility was performed with the use of E-test (bioMérieux, France). The EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints were used for interpretation with *Bacteroides fragilis* ATCC 25285 as a control. In the case of all three positive specimens obligatory anaerobic bacteria revealed significant presence. In the first sample, two anaerobic species were found, Gram-negative rods *B. fragilis* and Gram-positive cocci *Finegoldia magna.* In the second *B. fragilis* was isolated as a sole species. In the third sample which was taken from the hip *B. fragilis* together with *Staphylococcus epidermidis* were identified. MIC values for *B. fragilis* were as follows: penicillin G, 256 mg/L; amoxicillin/clavulanic acid, 0.75 mg/L, clindamycin, 0.125 mg/L, imipenem, 0.125 mg/L, metronidazole, 0.25 mg/L. On the basis of antimicrobial susceptibility results the antimicrobial therapy was changed to *i.v*. clindamycin 900 mg tid for 1 week, and then *p.o.* 300 mg qid for 4 weeks (5 weeks in total). The wound healed uneventfully and CRP level decreased to normal value.

Three months later the lumbar pain increased significantly and the sinus tract reopened. The patient was readmitted and the surgical debridement with the exchange of the fixation device and local application of collagen-gentamicin sponge with addition of vancomycin and ceftazidime was performed. Parenteral therapy consisted of *i.v.* clindamycin 900 mg tid and *i.v.* vancomycin, initially 1 g bid and then adjusted to achieve the therapeutic level in blood for 9 days. From two soft tissue samples collected intraoperatively from the implant (in sterile container and immediately, at the room temperature sent to the microbiological laboratory) the monoculture of clindamycin resistant *B. fragilis* (MIC = 256 mg/L) was obtained. It remained sensitive to ampicillin/sulbactam (MIC = 1.0 mg/L), piperacillin/tazobactam (MIC = 0.25 mg/L) and metronidazole (MIC = 0.25 mg/L). After 9 days, i.e., after achieving results of cultures therapy was switched from clindamycin and vancomycin to *p.o*. metronidazole 250 mg tid and *p.o.* ampicillin/sulbactam 750 mg bid and continued for 1 month. The wound eventually healed. Gradual improvement of laboratory parameters and the decrease of lumbar pain were observed. CRP dropped to 3.85 mg/L, WBC was 5650/μL and neutrophils were 57%. So far, after 2 years no recurrence of the infection has been observed.

Case 2

A 58-year-old man has been admitted to the orthopedic department with the diagnosis of the femur osteomyelitis with an active sinus of 2 months' duration with foul smelling purulent discharge. His medical history included a closed, high energy fracture of the femur (motor vehicle

collision) two years earlier, treated with open reduction and internal fixation complicated by infection and non-union. His other comorbidities included splenectomy performed after the accident, and gastrectomy performed years before due to peptic ulcers. On top of this the patient also turned out to be a heavy smoker. Upon admission the CRP was 18.7 mg/L, WBC 7.690/μL, and neutrophils were 55.2%.

Prior to the admission he had been treated for a month with oral rifampicin 300 mg bid and ciprofloxacin 500 mg bid for *S. epidermidis* cultured from the wound, but without any local signs of improvement.

The implant was removed and bone was stabilized with Ilizarov external fixator. Additionally acrylic cement segmental spacer loaded with vancomycin was implanted according to Masquelet technique.

Two swabs were taken intraoperatively from the wound, placed to the standard transport medium (Hagmed, Poland) and at once sent to the microbiological lab. The tests ware performed in accordance with routine procedures. The microbial culture method was used. Both clinical samples were incubated under aerobic and anaerobic conditions. The following media was used for the culture: Columbia agar +5% sheep blood, MacConkey agar, Sabouraud agar and Schaedler agar +5% sheep blood (bioMérieux, France). Presumably due to a prolonged antimicrobial therapy the samples taken intraoperatively were microbiologically negative and yet the histopathological assessment suggested infection. At the second stage performed 3 months later, the cement spacer was removed and internal bone transport was performed. The bone union, however, was not achieved. *S. epidermidis* was cultured from tissue samples. Inflammatory parameters were: CRP 88.5 mg/L, ESR 97/hour, WBC 9100/μL and neutrophils 84.5%. During the subsequent surgery the infected segmental bone defect was treated with implantation of vancomycin loaded allogenic bone grafts. The procedure turned out to be unsuccessful. Consequently after several months the removal of the non-healed bone grafts and debridement of the medullary canal with implantation of acrylic cement spacer loaded with vancomycin and meropenem was performed. The initial parenteral empiric treatment taking into account the previous presence of *S. epidermidis* consisted of 7 days *i.v.* rifampicin 300 mg bid and *i.v.* vancomycin initially 1 g bid and in turn adjusted to achieve the therapeutic level in the blood. Two samples, one from soft tissues and one from the marrow cavity were taken intraoperatively. The samples were cultured in vitro, bacteria were identified in the same manner as in the case 1described hereinabove. In both samples *E. faecalis* and *B. fragilis* were found. The results of susceptibility tests for *B. fragilis* consisting of the assessment of MIC value, were as follows: penicillin G, 16.0 mg/L; amoxycillin/clavulanic acid, 0.25 mg/L; clindamycin, 1.0 mg/L; imipenem, 0.06 mg/L; metronidazole, 0.25 mg/L. *E. faecalis* was susceptible to vancomycin but also to ampicillin. After initial 7-days treatment with rifampicin and vancomycin the antibiotic therapy was modified to *p.o.* ampicillin/sulbactam 375 mg tid for 4 weeks and the wound eventually healed. However, the patient's CRP reached a steady level of around 30 mg/L and several weeks after the discontinuation of oral antibiotics the discharge from the sinus was once again observed.

The patient underwent another limb-sparing procedure with removal of acrylic cement spacer from the cavitary and partially segmental bone defect, debridement and implantation of bone substituting material cerament (Bonesupport, Sweden). Intraoperative cultures from three tissue samples revealed the presence of *Proteus mirabilis* and *Enterobacter cloacae* with susceptibility of both pathogens to meropenem. No presence of previous pathogens i.e., *E. faecalis* and *B. fragilis* was shown. Therapy consisted of meropenem 2 g *i.v.* tid for 4 weeks supported by vacuum assisted closure (VAC) therapy of the residual wound. Normalization of CRP level, lack of clinical signs of anaerobic infection as well as progressive wound healing was observed.

Discussion

Orthopedic infections, especially those related to implants constitute a serious medical and socio-economic problem. Local immuno-incompetent zone around the implant, biofilm formation and poor local bioavailability of antibiotics may render the treatment long and unpredictable. The prolonged antibiotic therapy on the other hand may result in the emergence of the resistant strains. Thus, additionally complicating the therapy and requiring local administration of antibiotics as well as radical operative treatment [5].

Bacteria from the genus *Bacteroides* are well known as significant clinical pathogens. Among many other localizations they can infect bones and joints. Due to the complicated course of such infections requiring both surgery and long-lasting antibiotic therapy most of the affected patients require hospitalization. Many authors have elaborated on causative role of *B. fragilis* in osteomyelitis [1, 2, 6, 7], prosthetic joint infection [8, 9] and infections of native joints [1, 2, 10, 11]. Osteomyelitis caused by anaerobes is often a mixed infection, whereas most cases of arthritis involve one isolate [1, 2, 4, 8–10]. *B. fragilis* is usually responsible for late infections, manifesting its occurrence even 3 months after the trauma or surgery. Because of resistance to commonly used empiric treatment, it is often related to therapy of early aerobic infections. *B. fragilis* infection in most cases goes together with one or more of additional, local or systemic compromising factors like: diabetes, splenectomy, leukopenia,

hypogammaglobulinemia, alcoholism, drug abuse, neoplastic disease, immunosuppression, treatment with cytostatics and steroids, antimicrobial therapy [2, 4, 8, 11, 12]. Some of these risk factors were observed in both above described patients. The presence of implants as well as other risk factors in both patients favoured the bone infections and after success of initial therapy, recurrences caused by different, resistant to initial therapy microorganisms occurred. The incidence of anaerobic infections in orthopedics reported in the literature is often underestimated due to difficulties in culturing such bacteria. Pre-laboratory procedures are also critical for obtaining reliable test results. It is necessary to collect a contamination-free specimen. The specimen must be protected from oxygen during collection and transported to the laboratory immediately at room temperature. The identification of anaerobes is highly complex, and laboratories may use different identification systems. In addition, no growth of bacteria in vitro does not mean the absence of the pathogen in the side of infection. Unfortunately, most microbiology laboratories do not routinely identify anaerobes to species level or determinate their susceptibility as these procedures are costly and time consuming. Most clinical pathogens grow over 24 h in plate. In routine clinical microbiology anaerobic study with susceptibility test takes at least 5 days [13].

Although it is known, that anaerobes and especially Gram-positive anaerobic cocci such as *F. magna* are important etiological agents in these infections [3, 4, 14]. Unfortunately in most laboratories identification and susceptibility tests for anaerobes are not routinely performed. Patients are treated empirically on the basis of suspected etiology. The described above cases prove the necessity of culturing of anaerobes. In both cases first, empiric therapy was inadequate. In the case of the first patient described hereinabove (case 1) with implant related infection of the lumbar spine we have noticed that clindamycin therapy for 5 weeks resulted in a short term improvement. Three months later, however, a recurrence of infection appeared. In all three samples taken from the patient *B. fragilis* resistant to clindamycin was identified in monoculture. The therapy succeeded with ampicillin/sulbactam for 4 weeks. Clindamycin failure and subsequent clindamycin resistance of the isolate were likely due to inadequate time of therapy (Infectious Diseases Society of America; IDSA guideline recommends 6–8 weeks). On the other hand a prolonged therapy favours a development of resistance and the 1 month of ampicillin/sulbactam course was sufficient.

The second patient (case 2) had a mixed, anaerobe/aerobe infection caused by *E. faecalis* and *B. fragilis*. The therapy with ampicillin/sulbactam for 5 weeks has also proved effective in term of eradication *E. faecalis* and *B. fragilis*. But in spite of eradication of these pathogens with ampicillin/sulbactam a new superinfection with *P. mirabilis* and *E. cloacae* developed in the presence of cement spacer. Both isolates were resistant to ampicillin/sulbactam.

In a successful infection therapy, adequate identification and determination of the susceptibility of the pathogen to antibiotics are essential as well as an appropriate surgical debridement [1, 15].

Compliance with ethical standards

Conflicts of interest All authors declared that they have no competing interests.

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Informed consent Written informed consent was obtained from the patients for publication of this case report.

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