

CASE REPORT

Early aetiological agent identification of osteomyelitis with the Light Cyler SeptiFast test from peripheral blood

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SUMMARY

The commercial real-time PCR SeptiFast test allows identification of 25 bacterial and fungal species directly from peripheral blood in patients with bloodstream infections. A 22-year-old Caucasian woman reported an open femur fracture requiring a two step surgical procedure. After the second procedure the patient manifested sepsis and *Enterobacter cloacae/aerogenes* DNA was detected in the peripheral blood with the SeptiFast. Thereafter, femur osteomyelitis was diagnosed and *E cloacae* was cultured from intraoperative bone samples. The patient completed a 3-month course of antimicrobial therapy. Five months after therapy was discontinued, the patient remained symptom free, but delayed bone consolidation occurs. Molecular methods to identify aetiological agents in blood, in addition to conventional cultures, could represent an advantageous tool in clinical practice also in patients with post-traumatic bone infections.

BACKGROUND

The commercial real time PCR SeptiFast (SF) test (Light Cyler SeptiFast test, Roche Diagnostics, Mannheim, Germany) allows the identification of 25 different bacterial and fungal species directly in peripheral blood samples from patients with bloodstream infections.¹⁻³ The test has been extensively investigated and in a recent literature review, including 34 studies and 6012 patients with suspected sepsis, its high rule-in value for early pathogen detection was highlighted.⁴

Open fractures are reported to be at a high risk for infection caused by Gram-negative bacteria and staphylococci.⁵⁻⁸

This is a case of *Enterobacter cloacae* femoral osteomyelitis following a trauma. *E cloacae* was first identified from peripheral blood with the SF test and subsequently it was isolated from conventional cultures of intraoperative bone samples.

CASE PRESENTATION

A 22-year-old, Caucasian woman was admitted with an open, comminuted, supracondylar fracture of the right femur caused by a motorcycle accident. The day after admission the patient underwent open surgical reduction. Specifically, after repeated thorough irrigation, debridement and alignment of the femoral segments, external fixators were positioned and the wound was closed. Perioperative antimicrobial prophylaxis consisted of teicoplanin 400 mg day plus gentamicin 80 mg a day.^{9 10} Both

antimicrobials were continued after surgery for 10 days. During the same period the patient was also receiving paracetamol and ketorolac to control the pain and enoxaparin 4000 U day. At the first admission, laboratory examinations evidenced white blood cell (WBCs) counts 12 530 mm³ with 82.2% neutrophils, haemoglobin (Hb) 11.2 g/dL, and normal liver and renal functional parameters. The day after, WBC results were normal, while Hb dropped to 8.5 g/dL. During the 10 days of hospitalisation, the patient presented a persisting low-grade fever without clinical or laboratory evidence of either surgical or other site infection. Two weeks after discharge, the patient was re-admitted to exchange from external to internal fixation. On admission, the patient reported that, while at home, she had pain at the surgical site and occasionally her body temperature had been above 38°C. For this reason the patient had been prescribed cefixime 400 mg twice a day for the last week. On admission, no clinical evidence of infection of the wound or the fixation entrance was observed; WBCs were 7370 mm³ with 61.5% neutrophils and Hb was 10.7 g/dL. The patient underwent surgery which consisted of osteosynthesis of the fracture with an internal plate and screws. Three full femoral cerclages were also placed. Perioperative prophylaxis consisted of cefazolin 2 g before surgery.^{9 10} On the following days, the patient had low-grade fever, the right leg and knee were oedematous, erythematous and painful while WBCs, which were increased the day after surgery, returned to normal value. These findings were referred as due to surgery, however, on the sixth day after surgery, the temperature reached 39°C with clinical evidence of systemic inflammatory response syndrome.¹¹

INVESTIGATIONS

At this time, WBCs were 10 930 mm³, neutrophils 88.2%, C reactive protein (CRP) 14.3 g/dL (normal value ≤0.5 mg/dL), erythrocyte sedimentation rate (ESR) 76 mm 1 h and liver and renal function tests were normal. Chest radiography results were negative, and urine and blood cultures were reported negative as well. The SF test from peripheral blood was positive for *E cloacae/aerogenes*.

Subsequently, *E cloacae* was isolated from deep wound secretion twice, 9 and 14 days after the SF result. Both isolates resulted resistant to amoxicillin/clavulanic acid, cefuroxime, cefixime and



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susceptible to imipenem, ceftriaxone, gentamicin and ciprofloxacin.

DIFFERENTIAL DIAGNOSIS

They consisted of superficial surgical site infection, post-traumatic osteomyelitis, post-surgical healthcare-associated osteomyelitis, nosocomial osteomyelitis and infection at sites remote from the operative field.

TREATMENT

After obtaining blood and urine cultures together with a blood sample for the SF test and a chest radiography, vancomycin 1 g twice a day and imipenem 500 mg four times a day were administered. Two days later, the fever decreased, WBCs were reduced to 7900 mm³ (63.5% neutrophils), CRP 7.8 mg/dL and ESR 75 mm 1 h. Body temperature was normal by day 5. After another week, while the patient remained without fever and leg pain and inflammatory markers continued to decrease, dehiscence of the surgical wound with purulent discharge occurred, yielding *E. cloacae* growth. Vancomycin was suspended, and ciprofloxacin 750 mg twice a day was added to imipenem. One week later, the patient underwent a surgical debridement of the infected area of the femur. From intraoperative bone samples, *E. cloacae* was cultured, with it having an antimicrobial susceptibility pattern identical to the previous isolates. The patient completed a 3-month course of therapy with imipenem 500 mg four times a day and ciprofloxacin 750 mg twice a day for 12 days, followed by ceftriaxone 2 g a day and ciprofloxacin 500 mg twice a day for 46 days, and then ciprofloxacin 500 mg a day for 30 days.

OUTCOME AND FOLLOW-UP

Five months after therapy was discontinued, the patient remained symptom free, and ESR, CRP and WCCs are in the normal range while the radiological study of the femur shows a delayed bone consolidation.

DISCUSSION

This is a case of *E. cloacae* femur osteomyelitis following an open fracture of the femur. In this case the pathogen was first identified from peripheral blood with the SF test, and subsequently from cultures of purulent wound discharge and intraoperative bone samples.

E. cloacae is a Gram-negative rod from the Enterobacteriaceae family. Although many of these microorganisms are found in the lower gastrointestinal tract of various animals, they can also be found in the environment, for example in water and in soil.^{12 13} *Enterobacter* spp. are common hospital pathogens, but community-acquired infections do also occur.^{12 13} With regard to the case reported, one can postulate that *E. cloacae* was inoculated into the bone at the time of trauma due to soil contamination, or it was acquired in the hospital as a consequence of the first or the second surgical intervention or even at home between the two surgical procedures. From the clinical course and the available laboratory data, it is not possible to time exactly when the bone infection occurred. In fact, intraoperative cultures were not obtained during surgery for internal fixation as surgical site infection was not suspected by the operating surgeon. It is plausible that tissue contamination occurred at the time of trauma and was not cleared despite toilette and antimicrobial prophylaxis^{8 12} causing a subclinical bone infection. Moreover, although the isolate, *in vitro*, resulted resistant to cefixime,¹⁴ therapy with a high dose of this antibiotic could have contributed to shade the clinical picture. On the other

hand, if femoral contamination occurred during the surgical procedures, *Staphylococcus aureus* rather than *E. cloacae* would be expected as the most probable infection agent.^{9 10} It has been reported that bone infections due to Gram negatives, including Enterobacteriaceae, are often secondary to environmental contamination of traumatic wounds.⁷⁻⁹

One of the advantages of molecular-based detection systems is that they can allow the identification of microbial DNA in patients receiving antimicrobial therapy detecting also non-viable microorganisms.¹⁻⁴ In addition, the results of this test, performed immediately after blood sample collection, can be available to the clinicians in less than 6 h, thus allowing early appropriate empiric therapy.¹⁻⁴ In this case, the SF result was available over 1 week before culture results, and the detection of *E. cloacae* DNAemia was the first laboratory result for *E. cloacae* osteomyelitis suspicion. This finding justified the administration of imipenem in addition to vancomycin. On the other hand, the advantage of a culture is that a susceptibility test of an isolate can be performed.² In the reported case, the isolation of the pathogen confirmed the clinical significance of *E. cloacae/aeerogenes* DNA detection and its susceptibility to imipenem.

To our knowledge, this is the first report in which *Enterobacter* osteomyelitis has been diagnosed by a molecular test from peripheral blood, before clinical signs of infection became evident. Figueroa *et al*¹⁵ by means of SF assay, reported a diagnosis of *E. cloacae/aeerogenes* fatal sepsis associated with lung infiltrates in a pregnant woman. Unlike our case, the molecular test was used late in the diagnostic work-up with delayed appropriate antimicrobial treatment.

Learning points

- ▶ Molecular-based diagnostic methods from peripheral blood could represent an advantageous tool in addition to conventional cultures also in post-traumatic bone infections.
- ▶ Detection of aetiological agent(s) at the site of the infection is/are crucial for effective therapy.
- ▶ Microbiological investigations need to be obtained also when infection is not suspected on clinical findings.

Contributors MBP and GR were involved with patient care, data collection and manuscript drafting, CL and AM were involved with microbiological investigations and manuscript drafting.

Competing interests None.

Patient consent Obtained.

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