Journal of Medical Microbiology (2011), **60**, 1046–1049 DOI 10.1099/jmm.0.026351-0

Case Report

Four cases of bacteraemia caused by *Fusobacterium nucleatum* in febrile, neutropenic patients

Gabriella Terhes,[1] Klára Piukovics,[2] Edit Urbán[1] and Elisabeth Nagy[1]

[1]Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary
[2]2nd Department of Internal Medicine, University of Szeged, Szeged, Hungary

Correspondence
Gabriella Terhes
terhesga@freemail.hu

Received 21 September 2010
Accepted 10 March 2011

Although bacteraemia caused by obligate anaerobic bacteria is a rare event, this phenomenon will be an emerging problem among oncohaematological patients. We report four cases of bacteraemia caused by *Fusobacterium nucleatum* in febrile, neutropenic patients over a 10 month period. All patients had haematological malignancy and severe neutropenia, and three of them suffered from oral mucositis or oedema of the oral mucosal surfaces, which was the probable portal of entry. All isolated strains were susceptible to standard anti-anaerobic antibiotics.

Introduction

Bacteraemia caused by anaerobic bacteria is a rare event; its incidence is about 0.5–9 % of all positive blood cultures (James & Al-Shafi, 2000; Ortiz & Sande, 2000; Goldstein, 1996). In spite of this low incidence, in most of the cases it is associated with high mortality, because of the delay in starting adequate treatment, and the inconsistent use of anaerobic culture methods. Naturally its outcome is also correlated with the patient's age and underlying disease. Since anaerobes are a predominant part of the normal commensal flora of mucous membranes, the majority of infections caused by these bacteria are of endogenous origin. When the oral cavity, the intestine or the female genital organs are the source of these infections, mixed anaerobic bacteria may be the predominant isolates from blood cultures (Brook, 2002). Bacteraemia caused by fusobacteria is characteristic in neutropenic patients; however, this organism is not a frequent causative agent of septicaemia (Candoni *et al.*, 2003; Fanourgiakis *et al.*, 2003).

During a 10 month period, four cases of bacteraemia caused by *Fusobacterium nucleatum* were detected in patients with haematological malignancies in the Haematology Unit, University Hospital of Szeged, Hungary. Our aims were to analyse retrospectively the clinical background and the microbiological characteristics of these cases, to draw attention to the importance of the regular use of anaerobic blood culture bottles, the necessity of the early recognition of anaerobic bacteraemia and the use of anti-anaerobic antibiotics as part of the empiric therapy.

Abbreviation: WHO, World Health Organization.

Case report

Case 1

A 51-year-old woman was admitted to the Haematology Unit with acute myelomonocytic leukaemia; therefore, cytosine arabinoside (2200 mg per day), mitoxantrone (15 mg per day) and etoposide (220 mg per day) therapies were given over 5 days. At the end of the chemotherapy, the patient had developed fever and oropharyngeal mucositis of World Health Organization (WHO) grade 3 (ulcers, liquid diet is required) (Miller *et al.*, 1981). Routine laboratory investigations revealed that the white blood cell and neutrophil counts were 5500 cells mm^{-3} and 100 neutrophils mm^{-3}, respectively. During the febrile episode, blood samples for cultures were drawn through a central venous catheter (two pairs of aerobic and anaerobic bottles) and the peripheral vein (two pairs of aerobic and anaerobic bottles); after that, empiric antibiotic treatment, levofloxacin (500 mg once a day), was started. On the basis of routine laboratory procedures, blood cultures were sent immediately to the laboratory, where they were incubated in a BACTEC blood culture system (BD BACTEC 9120; BD Diagnostic Systems) until a positive signal was generated. If a positive signal appeared, in the case of the aerobic bottle, blood was inoculated onto the surface of aerobic agar plates (sheep blood agar, chocolate agar, eosin methylene blue and Sabouraud agar plates); while in the case of the anaerobic bottle, besides aerobic agar plates, Columbia agar base (Oxoid) supplemented with 5 % (v/v) cattle blood, haemin and vitamin K_1 was also inoculated. For aerobic bacteria, the plates were incubated at 37 °C in a 5 % CO_2-containing environment for 24 h or in ambient air. For the isolation of anaerobic bacteria, the anaerobic plate was incubated in an atmosphere of 90 % N_2, 5 % H_2 and 5 %

CO_2 (Bactron chamber; Sheldon Manufacturing). After getting a positive signal, phase-contrast microscopy and Gram staining were performed, and on the basis of the microscopy findings, direct susceptibility testing was set up for aerobic bacteria. In the cases of the two aerobic blood culture bottles collected by venepuncture and through the central venous catheter, positive signals were generated, and after 24 h of incubation *Staphylococcus haemolyticus* grew in these bottles. On the basis of antibiotic susceptibility results, the patient was treated with teicoplanin (daily dose 400 mg) for 14 days. At the same time, after 50 and 77 h of incubation for the anaerobic blood culture bottles collected by venepuncture and through the central venous catheter, positive signals were obtained. Phase-contrast microscopy examination showed non-motile, slender rods with pointed ends, which proved to be Gram-negative by staining. On the basis of these findings, the possibility of *Fusobacterium* sp. arose; therefore, metronidazole therapy (500 mg three times a day) was started. Colonies on the anaerobic blood agar plate supplemented with 1 mg vitamin K_1 l^{-1} and 0.3 g cysteine l^{-1} were irregular with a flecked appearance. The organism did not show growth in the presence of 20 % bile. Carbohydrate fermentations and lipase production were negative, while the isolated strain produced indole. The strain was identified as *F. nucleatum* using commercial biochemical tests. Antibiotic susceptibility testing was performed using the Etest method (AB Biodisk), and the strain was susceptible to penicillin, amoxicillin/clavulanic acid, cefoxitine, imipenem, metronidazole and clindamycin. Two weeks later, after remission, the patient was discharged home.

Case 2

A 71-year-old woman, who suffered from acute myeloid leukaemia, was admitted to the University Hospital of Szeged with a febrile episode, mucositis WHO grade 1 (soreness and erythema) and the possibility of perianal abscess. Laboratory investigations showed neutropenia and a high C-reactive protein level. Chest radiography revealed pulmonary infiltration in the upper right side of the lung. Blood culture samples (aerobic and anaerobic bottles) were taken from the peripheral vein, and empiric antibiotic therapy, piperacillin/tazobactam (4.5 g three times a day), was started. Blood culture bottles were processed according to the above-mentioned routine laboratory procedures. There was no evidence of perianal abscess by surgical examination and after 47 h of incubation, phase-contrast microscopy examination from the positive anaerobic blood culture bottle showed very slender, long, non-motile rods. Species level identification and antibiotic-susceptibility testing were performed as described above. In spite of the *in vitro* susceptibility of the isolated strain to piperacillin/tazobactam, because of persistent fever, 5 days later the antibiotic therapy was changed to meropenem (1 g three times per day). After this, the patient became afebrile and she was discharged home.

Case 3

A 46-year-old man was diagnosed with acute pre-B-cell lymphoblastic leukaemia. In his second remission, after consolidation therapy (high dose cytosine arabinoside and mitoxantrone), he had developed fever and neutropenia without mucositis despite the chemotherapeutic treatment. Blood samples (aerobic and anaerobic bottles) for culture were taken according to routine procedures, and the anaerobic blood culture bottle gave a positive signal after 35 h of incubation. On the basis of findings of the microscopic examination, the possibility of bacteraemia caused by *Fusobacterium* sp. arose and empiric antibiotic therapy, piperacillin/tazobactam (4.5 g three times per day), was started. Species level identification and antibiotic susceptibility testing were performed. On the 11th day of adequate antibiotic therapy, the patient was discharged home.

Case 4

A 61-year-old woman with acute myeloid leukaemia was admitted to the Haematology Unit because of fever, cough and dyspnoea. Her C-reactive protein level was elevated, and her absolute neutrophil count was 200 neutrophils mm^{-3}. She had severe oropharyngeal mucositis of WHO grade 3, while chest radiography showed pulmonary infiltration on the left side of the lung. Blood culture samples (aerobic and anaerobic bottles) were taken and after that empiric antibiotic therapy, ciprofloxacin (200 mg twice a day) was started because of persistent fever. After 87 h of incubation in the case of anaerobic bottle, we got a positive signal. On the basis of the findings of the microscopic examination, amoxicillin/clavulanic acid (1.2 g every 12 h for 10 days) was started, and *F. nucleatum* grew from the positive blood culture bottle. On the 30th day of hospitalization, the patient was discharged home.

Microbiological investigations

During the febrile period, at least one pair of blood samples was taken in aerobic and anaerobic bottles from all patients. Blood culture bottles were placed immediately in the BACTEC system (BACTEC 9120; BD Diagnostic Systems), and incubated at 37 °C until a positive fluorescence signal was detected. When positive signals were generated after 35–87 h of incubation, phase-contrast microscopy examination and Gram-staining preparation were performed and subcultures were plated on anaerobic blood agar plates supplemented with 1 mg vitamin K_1 l^{-1} and 0.3 g cysteine l^{-1} for the isolation of anaerobic bacteria. For the isolation of aerobic bacteria, sheep blood agar, chocolate agar, eosin methylene blue and Sabouraud agar were inoculated. Anaerobic plates were placed in an anaerobic chamber (Bactron; Sheldon Manufacturing) for 48 h at 37 °C, while the aerobic plates were incubated at 37 °C, for 24 h in 5 % CO_2, and in ambient air. Identification of the isolated strains was carried out according to

the Wadsworth Manual (Jouseimies-Somer *et al.*, 2002) [e.g. special-potency discs, use of BBE (bacteroides bile aesculin agar) (for the detection of growth in the presence of bile) and EYA(egg yolk agar) (for the detection of lipase activity) media] and using API Rapid ID 32A (bioMérieux). We also checked the cellular and colony morphologies, fluorescence under UV light and greening of blood agar after exposure to air. Determination of the MICs for penicillin, amoxicillin/clavulanic acid, cefoxitine, imipenem, metronidazole and clindamycin was carried out using the Etest method (AB Biodisk) and the results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2007).

Discussion

Microbiological investigation revealed the presence of *F. nucleatum* in the blood cultures of the four patients. In three cases, only one anaerobic bottle gave a positive signal, while in one case, two aerobic blood culture bottles collected at the same time were positive for coagulase-negative staphylococci. All patients had clinical symptoms of sepsis; since the isolated *F. nucleatum* is not a member of the skin flora, its role as a possible contaminant was excluded. The mean number of days required for the blood culture bottles to become positive was 2.6 days (range 35 to 87 h). All four *F. nucleatum* strains proved to be susceptible to all tested anti-anaerobic antibiotics (penicillin, amoxicillin/clavulanic acid, clindamycin, cefoxitin, imipenem and metronidazole). In all cases, the underlying disease was haematological malignancy, acute myeloid or lymphoblastic leukaemia. Two patients (cases 1 and 3) received chemotherapy before the development of a febrile period, one of them had confirmed oropharyngeal mucositis, while in the second case, there were no clinical symptoms of this, but the therapy used is known to make patients susceptible to the development of oral mucositis. In case 4, the patient had severe oropharyngeal mucositis without previous chemotherapy, while in case 2, the peritonsillar region and the soft palate were oedematous.

Fanourgiakis *et al.* (2003) showed 15 cases of bacteraemia caused by *Fusobacterium* spp. over a 6.5 year period. Among these, 13 patients were neutropenic, their mean age was 53 years, and the majority of them had underlying haematological malignancies and oral postchemotherapy mucositis. As oral mucositis could be observed in almost all patients, this was the probable source of the bacteraemia. This study also demonstrated that a relatively long incubation period (about 5 days) was sometimes necessary for getting positive signals in the case of anaerobic bottles.

On the basis of literature data, between the 1960s and 1970s, the incidence of anaerobic bacteraemia increased due to improvements in anaerobic laboratory culture methods, later this was followed by a decrease, while between 1993 and 2004, the mean incidence of anaerobic bacteraemias increased again (Ortiz & Sande, 2000; Dorsher *et al.*, 1991; Lassmann *et al.*, 2007). The majority of patients with anaerobic bacteraemia had predisposing factors, such as severe neutropenia, chemotherapy-induced oral mucositis or other types of oral lesions (Landsaat *et al.*, 1995; Vidal *et al.*, 2000). Because of these, prophylaxis with anaerobic coverage for postchemotherapy neutropenia should be reasonable according to some publications; however, the use of prophylaxis may contribute to the selection of resistant strains from the indigenous flora, and therefore an optimal decision is very difficult (Fanourgiakis *et al.*, 2003; Landsaat *et al.*, 1995).

In 2008, Goldstein and colleagues showed that laboratories that routinely performed anaerobic cultures, processed mainly blood and wound cultures for anaerobes, at the same time, species level identification and antimicrobial susceptibility testing needed improvement (Goldstein *et al.*, 2008). As a consequence of limited susceptibility data for this topic, clinicians frequently apply broad-spectrum antibiotics; this may lead to the development of new resistance mechanisms in anaerobic bacteria, the outcome of infections caused by anaerobes may worsen, mainly if the patient is not treated with the appropriate antibiotics, and the emergence and spread of resistant strains may increase the financial burden on health care (Goldstein *et al.*, 2008; Hecht, 2007). The importance of adequate therapy for anaerobic bacteraemia was also shown by Salonen *et al.* (1998). They examined the incidence of anaerobic bacteraemia and mortality. If the patient received adequate antimicrobial therapy before the results of blood cultures, the mortality was 18 %. If ineffective antimicrobial therapy was changed to effective on the basis of culture results, the mortality proved to be 17 %, while if the patient was treated with ineffective antibiotics the mortality reached 55 % (Salonen *et al.*, 1998). In many cases, to reduce the costs of microbiological investigations, there is no routine performance of anaerobic blood culture. Clinicians request anaerobic blood culture only in special cases, such as during surgical interventions or in the case of certain patients groups (e.g. haematological patients). On the basis of the work by Rosenblatt (1997), the presentation of anaerobic infections is frequently atypical and originates from unsuspected sources; thus, development of these is unpredictable. This observation also confirmed the routine use of anaerobic blood cultures (Rosenblatt, 1997). The importance of the routine anaerobic blood cultures is also emphasized by the fact that the use of anaerobic bottles may increase the yield of both obligate and facultative anaerobes, including streptococci (Rosenblatt, 1997).

Because of the aggressive treatment of haematological patients, mucositis commonly occurs with consequent translocation of commensal flora members; thus, in this patient group, we need to think about anaerobes as causative agents of bacteraemia and anti-anaerobic coverage in empiric therapy is desirable. As a consequence of the emergence of highly virulent strains and the increasing frequency of resistant isolates, the routine use of anaerobic blood culture is a reasonable choice.

References

Brook, I. (2002). Clinical review: bacteremia caused by anaerobic bacteria in children. *Crit Care* **6**, 205–211.

Candoni, A., Filì, C., Trevisan, R., Silvestri, F. & Fanin, R. (2003). *Fusobacterium nucleatum*: a rare cause of bacteremia in neutropenic patients with leukemia and lymphoma. *Clin Microbiol Infect* **9**, 1112–1115.

CLSI (2007). *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*, approved standard, 7th edn, M11–A7 (ISBN 1–56238–626–3). Wayne, PA: Clinical and Laboratory Standards Institute.

Dorsher, C. W., Rosenblatt, J. E., Wilson, W. R. & Ilstrup, D. M. (1991). Anaerobic bacteremia: decreasing rate over a 15-year period. *Rev Infect Dis* **13**, 633–636.

Fanourgiakis, P., Vekemans, M., Georgala, A., Daneau, D., Vandermies, A., Grenier, P. & Aoun, M. (2003). Febrile neutropenia and *Fusobacterium* bacteremia: clinical experience with 13 cases. *Support Care Cancer* **11**, 332–335.

Goldstein, E. J. (1996). Anaerobic bacteremia. *Clin Infect Dis* **23** (Suppl. 1), S97–S101.

Goldstein, E. J. C., Citron, D. M., Goldman, P. J. & Goldman, R. J. (2008). National hospital survey of anaerobic culture and susceptibility methods: III. *Anaerobe* **14**, 68–72.

Hecht, D. W. (2007). Routine anaerobic blood cultures: back where we started? *Clin Infect Dis* **44**, 901–903.

James, P. A. & Al-Shafi, K. M. (2000). Clinical value of anaerobic blood culture: a retrospective analysis of positive patient episodes. *J Clin Pathol* **53**, 231–233.

Jouseimies-Somer, H. R., Summanen, P., Citron, D. M., Baron, E. J., Wexler, H. M. & Finegold, S. M. (2002). *Wadsworth-KTL Anaerobic Bacteriology Manual*, 6th edn, June 2002. Belmont, CA: Star Publishing.

Landsaat, P. M., Van der Lelie, H., Bongaerts, G. & Kuijper, E. J. (1995). *Fusobacterium nucleatum*, a new invasive pathogen in neutropenic patients? *Scand J Infect Dis* **27**, 83–84.

Lassmann, B., Gustafson, D. R., Wood, C. M. & Rosenblatt, J. E. (2007). Reemergence of anaerobic bacteremia. *Clin Infect Dis* **44**, 895–900.

Miller, A. B., Hoogstraten, B., Staquet, M. & Winkler, A. (1981). Reporting results of cancer treatment. *Cancer* **47**, 207–214.

Ortiz, E. & Sande, M. A. (2000). Routine use of anaerobic blood cultures: are they still indicated? *Am J Med* **108**, 445–447.

Rosenblatt, J. E. (1997). Can we afford to do anaerobic cultures and identification? A positive point of view. *Clin Infect Dis* **25** (Suppl. 2), S127–S131.

Salonen, J. H., Eerola, E. & Meurman, O. (1998). Clinical significance and outcome of anaerobic bacteremia. *Clin Infect Dis* **26**, 1413–1417.

Vidal, A. M., Sarria, J. C., Kimbrough, R. C., III & Keung, Y. K. (2000). Anaerobic bacteremia in a neutropenic patient with oral mucositis. *Am J Med Sci* **319**, 189–190.