Original article

European Journal of Microbiology and Immunology 5 (2015) 3, pp. 199–204
DOI: 10.1556/1886.2015.00021

EVALUATION OF BLOODSTREAM INFECTIONS DURING CHEMOTHERAPY-INDUCED FEBRILE NEUTROPENIA IN PATIENTS WITH MALIGNANT HEMATOLOGICAL DISEASES: SINGLE CENTER EXPERIENCE

Klára Piukovics[1], Gabriella Terhes[2], Andrea Lázár[2], Flóra Tímár[1], Zita Borbényi[1], Edit Urbán[2,*]

[1]Second Department of Internal Medicine and Cardiological Center, University of Szeged
[2]Institute of Clinical Microbiology, University of Szeged

Received: July 28, 2015; Accepted: August 26, 2015

From year to year, it is important to get an overview of the occurrence of causative agents in febrile neutropenic patients to determine the empiric treatment. Thus our aims were to evaluate a four-year period regarding the prevalence of bloodstream infections and the most important causative agents. During this period, 1,361 patients were treated in our hematology ward because of various hematological disorders. 812 febrile episodes were recorded in 469 patients. At that time, 3,714 blood culture (BC) bottles were sent for microbiological investigations, 759 of them gave positive signal. From the majority of positive blood culture bottles (67.1%), Gram-positive bacteria, mainly coagulase-negative staphylococci (CNS), were grown. Gram-negative bacteria were isolated from 32.9% of the positive blood culture bottles, in these cases the leading pathogen was *Escherichia coli*. The high prevalence of CNS was attributed to mainly contamination, while lower positivity rate for Gram-negative bacteria was associated with the use of broad-spectrum empiric antibiotic treatment.

Keywords: sepsis, blood culture, neutropenia, malignancy, hematology

Introduction

Infectious complications, especially bloodstream infections (BSIs), are major causes of morbidity and mortality among patients who suffered from malignant hematological diseases and treated with intensive chemotherapeutic regimens [1, 2]. In these clinical settings, bacterial cultures from blood are of great diagnostic value and the gold standard to detect bloodstream infections; in addition to this, the results of blood cultures provide epidemiological data which are useful to determine empiric antibiotic therapy. However, the diagnosis of BSIs is still challenging in this patient group, because about half of all BSI cases are culture negative mainly because of the frequently used prophylactic antibiotics [3]. To overcome the inhibitory effect of antibiotics, special blood culture bottles containing resin have been developed; thus, modest increase in the sensitivity of culture has been achieved [3]. In the early 1970s, introducing empiric treatment protocols and antibiotic prophylaxis, increasing use of certain chemotherapeutic drugs associated with frequent oral mucositis, and frequent use of central venous catheters have changed the spectrum of pathogens in febrile neutropenic patients shifting it from Gram-negative to Gram-positive bacteria, especially viridans group streptococci and coagulase-negative staphylococci [3–6].

The aim of this study was to evaluate occurrence of bacterial species causing bloodstream infections due to febrile neutropenic episodes in the hematology ward of the University Hospital in Szeged, Hungary, between 2005 and 2008.

Materials and methods

Patients

Between 2005 and 2008, 469 patients with febrile neutropenia (230 females and 239 males, median age 60 years) were observed in our department with various hematological diseases. Collected data from patients' documentations included demographics of patients, diagnosis, febrile

* Corresponding author: Edit Urbán; Institute of Clinical Microbiology, University of Szeged, Semmelweis street 6, H-6725 Szeged, Hungary; E-mail: urban.edit@med.u-szeged.hu

ISSN 2062-8633 / $ 20.00

episodes, source of fever and source of infection, neutrophil count, and clinical significance of the isolated organism. Infectious complications were categorized into three groups: 1 – fever of unknown origin (FUO), 2 – microbiologically documented infection (MDI), and 3 – clinically documented infection (CDI).

Definitions

Febrile neutropenia was defined if a single oral temperature was measured higher than 38.3 °C, or temperature was 38.0 °C or higher for 1 h. Neutropenia was defined as absolute neutrophil count (ANC) less than 0.5×10^9/l or less than 1.0×10^9/l and rapidly declined below 0.5×10^9/l [7]. A single positive blood culture (BC) was considered significant if the isolated strain was clinically relevant cause of the infection. Common skin contaminants (CNS, propionibacteria) were considered significant only if they could be found in two consecutive BC samples or if there were concurrent skin, soft tissue, or catheter-related infections. BSI was defined as polymicrobial if more than 1 bacteria grew from BC on the same day. Microbiological results were retrieved from the Clinical Microbiological Laboratory information system. Medical database of patients was used to collect information on the hematologic diseases, presence of febrile neutropenic episode, duration of neutropenia, and source of infection.

Analysis of blood cultures

BC samples were taken at the onset of fever. In patients having central venous catheters, BCs were taken from both central and peripheral veins. For collection of blood culture, the blood culture system (BD Bactec, Beckton Dickinson, USA) including aerobic, anaerobic bottles, and bottles for fungi was used. After taking blood, bottles were immediately placed in an incubator, where these were incubated for 5–14 days depending on the type of the putative pathogens. In the case of positive signal produced by the instrument on the basis of bacterial or fungal growth, microscopic examinations (phase contrast microscopy and examination of Gram-stained preparations) and culture were performed. For aerobic culture, Columbia blood agar supplemented with 5% sheep blood (BioMérieux, Marcy l'Etoile, France), chocolate agar supplemented with PolyViteX (BioMérieux, Marcy l'Etoile, France), eosin methylene blue (Lab M, UK) and Sabouraud Chloramphenicol (Bio-Rad, France) agars, and, for anaerobic culture, Schaedler agar supplemented with 5% sheep blood (BioMérieux, Marcy l'Etoile, France) were inoculated with one drop of blood. Plates were incubated at 37 °C for 24 h in a 5% CO_2 incubator or 37 °C for 24 h under normal atmosphere or at 37 °C for 48 h in an anaerobic cabinet (Concept 400; Ruskinn Technology Ltd., Bridgend, UK) under a gas composition of 85% N_2, 10% H_2, and 5% CO_2. From pure culture, antibiotic susceptibility tests were performed on the basis of Clinical Laboratory Standard Institute recommendations [8–11].

Antibiotic protocol

At the onset of fever, after taking BC, broad spectrum antibiotics were started empirically (piperacillin–tazobactam, cefepime, and imipenem or meropenem). Antibiotic dosage was modified according to the patient's renal function. Patients were examined once daily by their physician to detect potential source of infection. After 48–72 h observations, the patient's condition was reevaluated. Changes in empiric antibiotic therapy depended on BC results and clinical response. In afebrile and culture-negative patients with stable clinical state, empiric antibiotic treatment was continued until ANC reached 500/μl. Vancomycin was used in patients with central venous devices, persistent fever, and hypotension [12]. On day 4–5, in patients with persistent fever suggesting fungal infection on the basis of clinical signs and computed tomography (CT) scans, amphotericin B was applied.

Results

During the four-year study period, 1,361 patients were treated in the hematology ward because of various hematological diseases. A total of 812 febrile episodes were recorded in 469 (34.5%) patients, and blood was collected for microbiological culture. Of the 469 patients, 128 (27.3%) had acute myeloid leukemia, 85 (18.1%) non-Hodgkin's lymphoma, 66 (14.1%) multiple myeloma, 64 (13.6%) chronic lymphocytic leukemia, 41 (8.7%) acute lymphoblastic leukemia, and 85 (18.1%) others (Hodgkin's lymphoma, myelodysplastic syndrome, chronic myeloprolipherative disorders etc.) *(Table1)*. Altogether, 3,714 blood culture bottles, 6.5 bottles/patient (ranging 2–12), were sent to the laboratory. In 126 (27%) of 469 patients, only one pair of blood culture bottles was taken by febrile episodes. Clinically documented infections could be observed in 430 of 812 febrile episodes (52.95%). The majority of them localized to the lung (39.5%). Colitis and skin and soft tissue infections were the second and third most common infections.

During the microbiological culture, 759 (20.4%) of 3,714 blood culture bottles gave positive signals. From the majority of positive blood culture bottles (509 bottles (67.1%)), Gram-positive bacteria were cultured. Among Gram-positive bacteria, the most frequent isolates were coagulase-negative staphylococci (65%), *Staphylococcus aureus* (10%), *Enterococcus* spp. (6.7%), *Popionibacterium acnes* (5.7%), β-hemolytic streptococci (3.1%), *Streptococcus pneumoniae* (2.8%), α-hemolytic streptococci (2.4%), *Clostridium* spp. (1.4%), and others (3%) (including *Listeria monocytogenes*, *Nocardia farcinica*, *Gemella* spp., *Micrococcus* spp., *Brevibacterium* spp., and Gram-positive nonidentified bacteria) *(Table 2)*. Gram-negative

bacteria were isolated from 250 (32.9%) blood culture bottles. High prevalence of *Escherichia coli* (52%) could be detected in these specimens, while 14% of samples contained *Pseudomonas aeruginosa,* 9.6% *Klebsiella* spp., 8% *Enterobacter* spp., 3.6% *Citrobacter* spp., 2% *Stenotrophomonas maltophilia,* 1.6% *Acinetobacter* spp., and 1.6% *Fusobacterium* spp. *(Table 3)*. Only six bottles proved to be positive for fungi during the examined period; in two cases, *Candida albicans* and also, in two bottles, *Candida tropicalis* could be detected, while two other bottles were positive for *Cryptococcus* spp.

Among Gram-positive isolates, coagulase-negative staphylococci (CNS) were identified in 331 cases. These blood culture samples were collected from 161 febrile episodes of 149 patients. In 50 febrile neutropenic episodes, CNS were relevant as a causative agent of fever because of the coexistence of skin, soft tissue, and central venous catheter-related infections. In the case of the remaining 111 cases, contamination could be the source of CNS.

Among rarely isolated pathogens, *Listeria* sp. was identified from one patient with acute myeloid leukemia (AML) due to second relapse in 2006. The patient had clinically documented pneumonia and was treated with ampicillin. In 2006 and 2008, *Burkholderia cepacea* was identified from two patients. One of them was observed with AML and did not receive any chemotherapeutic treatment. Blood culture sample was taken from peripheral vein. In this case, the patient was treated empirically with levofloxacin. The other patient suffered from relapsed Hodgkin lymphoma and was treated according to ESHAP chemotherapeutic regimen (combination of high-dose cytosine-arabinosid, methylprednisolone, cisplatin, and etoposide) through the central venous line. Two pairs of blood culture samples were taken due to febrile episode from catheter, and *B. cepacea* was grown from each samples. *N. farcinica* was isolated from a patient with large granular lymphocytic (LGL) leukemia. He underwent six cycles of combined chemotherapeutic treatment (cyclo-

Table 1. Patient characteristics at admission to hematology ward between 2005 and 2008

Patient characteristics	Number of patients *N* = 469 (%)
Age (median, years)	60
Gender	
Male	239 (51)
Female	230 (49)
Underlying diseases	
Acute myeloid leukaemia	128 (27.3)
Non-Hodgkin's lymphoma	85 (18.1)
Multiple myeloma	66 (14.1)
Chronic lymphocytic leukaemia	64 (13.6)
Acute lymphoblastic leukaemia	41 (8.7)
Others (chronic myeloprolipherative disorders, myelodysplastic syndrome, Hodgkin's lymphoma, etc.)	85 (18.1)

Table 2. Annually identified Gram-positive bacteria from blood culture

Isolated strain	Number of isolated strains according to years				Total *N* = 509 (%)
	2005	2006	2007	2008	
Coagulase-negative staphylococci	94	73	86	78	331 (65)
S. aureus	25	6	11	9	51 (10)
Enterococcus spp.	6	9	5	14	34 (6.7)
P. acnes	13	10	2	4	29 (5.7)
Beta-hemolytic streptococci	10	5	1	0	16 (3.1)
S. pneumoniae	5	0	0	9	14 (2.8)
Alfa-hemolytic streptococci	0	7	1	4	12 (2.4)
Clostridium spp.	2	4	0	1	7 (1.4)
Others (*L. monocytogenes, N. farcinica, Gemella* spp., *Micrococcus* spp., *Brevibacterium* spp., etc.)	4	6	2	3	15 (3)

Table 3. Annually isolated Gram-negative bacteria from blood culture bottles

Isolated strain	Number of isolated strains according to years				Total $N = 509$ (%)
	2005	2006	2007	2008	
E. coli	22	38	41	29	130 (52)
P. aeruginosa	6	6	10	13	35 (14)
Klebsiella spp.	0	10	6	8	24 (9.6)
Enterobacter spp.	7	3	0	1	20 (8)
Citrobacter spp.	2	3	2	2	9 (3.6)
S. maltophilia	0	0	2	3	5 (2)
Acinetobacter spp.	0	1	1	2	4 (1.6)
Fusobacterium spp.	0	2	2	0	4 (1.6)
Others (*H. influenzae, A. xylosoxidans, P. mirabilis, Salmonella* spp., *Neisseria* spp., Gram-negative non-identified rods, etc.)	5	4	5	5	19 (7.6)

phosphamide, vincristine, and prednisolone) and later had long term steroid therapy due to Coombs positive hemolytic anemia and active hemolytic events. On hospital admission, CT scan showed multiple lesions with perifocal oedema, but stereotactic core biopsy from lesions could not be performed because of the patient's severe clinical status. From one pair of blood culture, *N. farcinica* was isolated, but the patient died before adequate therapy could start. During the examined period, four cases of bacteremia caused by *Fusobacterium nucleatum* could be observed in this patient group. Two patients had acute myeloid leukemia, one patient had acute myelomonocytic leukemia, and one patient suffered from pre-B-cell lymphoblastic leukemia. Two patients received chemotherapy before positive blood culture; one of them had oral mucositis associated with the applied chemotherapy.

Discussion

Febrile neutropenia is the most important complication of chemotherapy in patients with hematologic malignancy. This may have influence on the applied chemotherapy; dose reduction or treatment delays can be observed frequently when febrile neutropenia is present, and this also has unfavorable long-term outcome in otherwise curable malignancy [13]. Bloodstream infections are among the most important bacterial infections, despite the development in the field of microbiological diagnosis and antimicrobial therapy; these infections are responsible for the large proportion of nosocomial infections worldwide. In the early 1960s, the importance of bloodstream infection in neutropenic patients had been recognized; thus, empirical treatment protocols were established for mainly Gram-negative bacteria [14]. Later, the spectrum of pathogens associated with BSI shifted from Gram-negative bacteria to Gram-positive bacteria due to the increased use of antibiotic prophylaxis and indwelling catheters allowing colonizations and infections with the skin flora. Nowadays, the most common pathogens isolated from blood are coagulase-negative staphylococci and various antibiotic-resistant bacteria. In the majority of cases, the source of these infections is unknown in spite of various efforts to find them. Recognition of changes in the epidemiology of BSIs is very important to modify the antibiotic policy because, on the basis of these findings, we can reduce the infection-related morbidity and mortality [15, 16]. During the four-year study period, the incidence of bacteremia was 20.4%. Similar findings were reported in the literature; Klastersky et al. showed that the incidence of bacteremia was 23% when they examined over two thousand patients with febrile neutropenia in cancer patients, while Viscoli et al. found that bacteremia occurred in 29% of patients with febrile neutropenia [17, 18]. Our findings correlated with the abovementioned literature data because, from the majority of blood culture bottles (13.7%), Gram-positive bacteria were isolated. Sixty-five percent of Gram-positive bacteria belonged to coagulase-negative staphylococci. However, in a study by Winston et al. in North America, Gram-negative bacteria (55.6%) [19] were responsible for the majority of bacteremia in febrile neutropenic patients. At the same time, other authors from Italy or France showed that the most important isolates in neutropenic patients are Gram-positive bacteria, including coagulase-negative staphylococci or streptococci, while Gram-negative organisms including *E. coli* or *Klebsiella* spp. and *P. aeruginosa* constitute smaller portion of the isolates [20, 21]. In our case, the most frequently used empirical treatment in this patient group is piperacillin–tazobactam or, if the patient has colitis or the possibility of abdominal infection is arisen, imipenem or meropenem is the frequently used antibiotic. Thus, the increased incidence of Gram-positive bacteria can be explained by the applied empirical antibiotic treatment, while the presence of coagulase-negative staphylococci could be attributed to the frequently used central venous catheters. The incidence of bacterial species in blood cultures can be influenced by the applied chemotherapy. In our case, 30% of patients with acute

leukemia received high-dose Ara-C chemotherapy, and 15% and 12% of patients were treated with fludarabin and Ara-C plus idarubicin, respectively. On the basis of literature data, increasing prevalence of Gram-positive cocci in febrile neutropenic patients could be observed after high-dose cytarabine chemotherapy; this was confirmed by our results [21]. Cordonnier et al. showed that the prevalence of staphylococci is higher than the prevalence of streptococci and enterococci in febrile neutropenic patients [21]. Similarly, our results confirmed this because, among Gram-positive bacteria, the majority of the isolated strains were coagulase-negative staphylococci; 6.7% and only 2.4% of Gram-positive bacteria belonged to *Enterococcus* spp. and β-hemolytic streptococci, respectively. A total of 331 blood culture samples proved to be positive for coagulase-negative staphylococci and were collected from 161 febrile episodes of 149 patients. In 50 febrile neutropenic episodes, coagulase-negative staphylococci were relevant pathogen of fever because of the coexistence of skin, soft tissue, and central venous catheter-related infection. The remaining 111 cases were supposed to be contamination.

From 32.9% of positive blood cultures bottles, Gram-negative bacteria were cultured; the majority of these proved to be positive for *E. coli* (52%). The second most common isolate was *P. aeruginosa* (14%), while the third one was *Klebsiella* spp. (9.6%). Similar findings by Ramphal could be found [16]. In this review, the results of four articles were analyzed, and, among Gram-negative organisms, the most important pathogens were also *E. coli*, *Klebsiella* spp., and *P. aeruginosa* [16].

Among rarely isolated bacteria, *Achromobacter xylosoxidans* and *Burkholderia cepacia* are usually associated with catheter-related sepsis, while *S. maltophilia* can cause mainly nosocomial bacteremia [22] and the possible source of *Haemophilus influenzae, Neisseria* spp., and *Gemella* spp. is the damaged oral mucosa. Because of the possible presence of unusual pathogens, such as anaerobic bacteria or fastidious microorganisms, the use of various blood culture bottles including anaerobic bottles should be considered.

Because of its rapid progression of infection in febrile neutropenic patients and difficulties in distinguishing infection from noninfected patients on the basis of clinical presentation in this patient group, the use of empirical antibiotic treatment is essential and may provide the possibility to reach better outcome.

References

1. Crawford J, Dale DC, Lyman GH: Chemotherapy-induced neutropenia: risks, consequences, and new direction for its management. Cancer 100, 228–237 (2004)
2. Madani TA: Clinical infections and bloodstream isolates associated with fever in patients undergoing chemotherapy for acute myeloid leukemia. Infection 28, 367–373 (2000)
3. Peters RPH, van Agtmael MA, Danner SA, Savelkoul PHM, Vandenbroucke-Grauls CMJE: New developments in the diagnosis of bloodstream infections. Lancet Infect Dis 4, 751–760 (2004)
4. Schimpff S, Satterlee W, Young VM et al.: Emipiric therapy with carbenicliin and gentamicin for febrile patients with cancer an granulocytopenia. N Engl J Med 284, 1061–1065 (1971)
5. Baden LR: Prophylactic antimicrobial agents and the importance of fitness. N Engl J Med 353, 1052–1054 (2005)
6. Hammond SP, Baden LR: Antibiotic prophylaxis for patients with acute leukemia. Leuk Lymphoma 49, 183–193 (2008)
7. Hughes WT, Armstrong D, Bodey GP et al.: Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Inf Dis. 34, 730–751 (2002)
8. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI document M100-S15 (ISBN 1-56238-556-9)
9. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S16 (ISBN 1-56238-588-7)
10. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI document M100-S17 (ISBN 1-56238-625-5)
11. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. CLSI document M100-S18 (ISBN 1-56238-653-0)
12. Mermel LA, Allon M, Bouza E et al.: Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. Clin Infect Dis 49, 1–45 (2009)
13. Keng MK, Sekeres MA: Febrile neutropenia in hematologic malignancies. Curr Hematol Malig Rep 8, 370–378 (2013)
14. Bodey GP. Buckley M, Sathe YS, Freireich EY: Quantitative relationship between circulating leukocytes and infections in patients with acute leukemia. Ann Intern Med 64, 328–340 (1966)
15. Syrjala H, Ohtonen P, Kinnunen U, Raty R, Elonen E, Nousiainen T, Jantunen E, Remes K, Itala-Remes M, Silvennoinen R, Koistinen P, Finnish Leukemia Group: Bloodstream infections during chemotherapy-induced neutropenia in adult patients with acute myeloid leukemia: treatment cycle matters. Eur J Clin Microbiol Infect Dis 29, 1211–1218 (2010)
16. Ramphal R: Changes in the etiology of bacteremia in febrile neutropenic patients and the susceptibilities of the currently isolated pathogens. Clin Infect Dis 39, 25–31 (2004)
17. Klastersky J, Ameye L, Maertens J, Georgala A, Muanza F, Aoun M, Ferrant A, Rapoport B, Rolston K, Paesamns M: Bacteremia in febrile neutropenic cancer patients. Int J Antimicrob Ag 30S, S51–S59 (2007)
18. Viscoli C, Cometta A, Kern WV et al.: Piperacillin–tazobactam monotherapy in high-risk febrile and neutropenic cancer patients. Clin Microbiol Infect 12, 212–216 (2006)
19. Winston DJ, Lazarus HM, Beveridge RA et al.: Randomized, double-blind, multicentre trial comparing clinafloxacin with imipenem as empirical monotherapy for

febrile granulocytopenic patients. Clin Infect Dis 32, 381–390 (2001)
20. Del Favero A, Menichetti F, Martino P et al.: A multicentre, double-bind, placebo-controlled trial comparing piperacillin–tazobactam with and without amikacin as empiric therapy for febrile neutropenia. Clin Infect Dis 33, 1295–1301 (2001)
21. Cordonnier C, Buzyn A, Leverger G et al.: Epidemiology and risk factors for gram-positive coccal infections in neutropenia: toward a more targeted antibiotic strategy. Clin Infect Dis 36, 149–158 (2003)
22. Picazo JJ: Management of the febrile neutropenic patient: a consensus conference. Clin Infect Dis 39, 1–6 (2004)