

Infections in Hematology

Infections in Hematology:

Modern Challenges and Perspectives

By

Igor Stoma and Igor Karpov

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TABLE OF CONTENTS

Author's Preface	vii
Chapter One.....	1
Hematopoietic stem cells transplantation and infectious complications: risk factors and etiology	
Chapter Two	13
Invasive pulmonary aspergillosis as a treatment challenge in hematology	
Chapter Three	21
Sepsis biomarkers in hematopoietic stem cells transplantation	
Chapter Four	35
Combination of sepsis biomarkers may indicate an invasive fungal infection	
Chapter Five	49
Infections originating from the gut microbiome and their role in modern hematology	
Chapter Six	57
Human herpesvirus 6 infections in hematology: unexpected consequences	
Chapter Seven.....	61
Pneumococcal vaccination in hematology: effects of implementation	
Chapter Eight.....	67
Graft-versus-host disease and infections in transplant recipients	
Chapter Nine.....	73
Mesenchymal stem cells transplantation and infections in hematology	
Chapter Ten	87
Reappraisal of selective oral decontamination in patients with hematological malignancies	

Chapter Eleven	101
Vaccination in patients with hematological malignancies	
Chapter Twelve	115
Cytomegalovirus infection in hematology: new agents are coming	
Chapter Thirteen	119
Tick-borne encephalitis as an endemic infection in immunocompromised hosts	
Chapter Fourteen	123
Epstein-Barr virus post-transplant lymphoproliferative disease	

AUTHOR'S PREFACE

*It is much more important to know
what sort of a patient has a disease than
what sort of a disease a patient has.*

William Osler

Recent advances in the treatment of hematological diseases have led to a marked increase in patient survival. However, as always, there is a fee for success and, in hematology, this charge appears in the form of an infection. This is why the efforts of many scientists and doctors today focus on studying infections in patients with immunosuppression. The trend towards specialization and the deepening of knowledge in modern medicine has led to the need for specialists in infections in immunocompromised hosts, and this book is for this area of medicine. We would like to express our gratitude to our colleagues and fellow specialists in the fields of hematology and infectious diseases, whose participation helped to create this book.

Igor Stoma, M.D., Ph.D.

CHAPTER ONE

HEMATOPOIETIC STEM CELLS TRANSPLANTATION AND INFECTIOUS COMPLICATIONS: RISK FACTORS AND ETIOLOGY

Bloodstream infections (BSI) remain an important cause of morbidity and mortality in recipients of hematopoietic stem cell transplantation (HSCT). They occur in 13.0–55.8% of all HSCT recipients with a mortality rate from 24.0 to 43.6% [1–6]. Although previously published studies provide some important information, the emergence of highly resistant Gram-negative pathogens requires new information about the risk factors and etiological spectrum of BSI in HSCT recipients. A knowledge of risk factors for a fatal outcome in patients with BSI after HSCT is one of the key steps when choosing the appropriate treatment regimen. There is a lack of published data about BSI in patients receiving HSCT and a poor knowledge of risk factors for adverse outcomes, which may be causes of inadequate empirical antibacterial therapy. This study was conducted to assess the risk factors for fatal outcomes and modern causes of BSI in HSCT recipients in the pre-engraftment period. A prospective observational study was performed to estimate possible risk factors for an adverse outcome in adult patients with a microbiologically-proven BSI in the pre-engraftment period following HSCT. Some of the indications for HSCT are acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, myelodysplastic syndromes, multiple myeloma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma.

This study was approved by the Scientific and Ethical Committees of Republican Center for Hematology and Bone Marrow Transplantation in Minsk, Republic of Belarus.

All-cause 30-days mortality after the onset of febrile neutropenia was a primary outcome in the study. The covariates in the analysis were age and

gender characteristics; type of HSCT (autologous/allogeneic); conditioning chemotherapy regimen type (myeloablative/non-myeloablative or reduced intensity); primary diagnosis; level of neutropenia when the first positive blood culture is collected; isolation of the carbapenem-resistant non-fermenting Gram-negative bacteria (*P. aeruginosa* and *A. baumannii*); isolation of the ESBL-producing member of *Enterobacteriaceae spp.* family; isolation of methicillin-resistant *S. aureus* (MRSA); adequacy of empirical antibacterial therapy. Epidemiological, clinical, and laboratory data were prospectively collected in each patient aged 18–70 years undergoing HSCT from January 2013 to October 2015. Among the exclusion criteria were: concurrent active oncological disease; hepatitis B or hepatitis C infection; active fungal disease; rheumatological diseases; and diabetes mellitus. When there was a possible active CMV infection (monitored by real time quantitative polymerase chain reaction), the patient was also excluded from the study. All patients had a complete clinical and hematological remission of the main disease at the start of HSCT. Blood cultures were obtained with standard precautions from all patients who fulfilled the criteria of febrile neutropenia in the pre-engraftment period after HSCT; identification and in vitro antibiotic susceptibility testing was also performed. Every patient with BSI was followed for at least 30 days after collecting the first positive blood culture. Only the first bacteremia episodes were included in the analysis.

Empirical antimicrobial therapy was defined as adequate if it was administered <24 hours after the collection of blood cultures and if the subsequently isolated microorganism was susceptible in vitro to at least one of the antibiotics administered. Empirical antimicrobial therapy was defined as inadequate if the subsequently isolated microorganism was in vitro resistant; intermediately susceptible to all of the administered antibiotics; the empiric antibacterial therapy was administered >24 hours after the collection of blood cultures; or the dosing regimen did not agree with the standard dosing recommendations. 30-days mortality was defined as the number of patients with BSI who died in a period of 30 days after the onset of febrile neutropenia divided by the total number of patients with BSI. An adverse outcome was defined as a death within 30 days from the onset of febrile neutropenia. The pre-engraftment period was defined as a period from day 0 to day 30 after HSCT [7]. BSI was defined as having a microbiologically proven growth from a blood culture of a patient after HSCT with febrile neutropenia. The criteria for febrile neutropenia was single oral temperature measurement of >38.3°C or a temperature of >38.0°C sustained over a 1-h period in a patient with an

absolute neutrophil count (ANC) of <500 cells/ μL or an ANC that is expected to decrease to <500 cells/ μL during the next 48 hours [8].

Transplantation was performed according to institutional protocols. Briefly, the most frequent myeloablative conditioning regimens were busulfan and cyclophosphamide (BuCy), as well as cyclophosphamide and total body irradiation (Cy+TBI). Non-myeloablative and reduced intensity conditioning mainly included fludarabine with melphalan, or treosulfan, and the BEAM regimen (carmustine, etoposide, cytarabine, melphalan). GVHD prophylaxis regimens included cyclosporine, methotrexate, and tacrolimus. Anti-thymocyte globulin was administered in cases of unrelated donors. Standard antibacterial prophylaxis in the department was based on fluoroquinolones (mainly ciprofloxacin 0.5g BID orally), which starts at the beginning of the conditioning regimen until the level of neutrophils in peripheral blood exceeds 500 cells/ μL . No routine antibacterial prophylaxis against *Streptococcus pneumoniae* was administered. Antifungal prophylaxis with fluconazole was prescribed to patients undergoing autologous HSCT and micafungin was used as antifungal prophylaxis in patients undergoing allogeneic HSCT. Prophylaxis against *Pneumocystis jirovecii* with trimethoprim-sulfamethoxazole was administered to all patients until the immunologic recovery after HSCT. Prophylaxis for infections caused by the herpes virus was performed by acyclovir. Real time quantitative polymerase chain reaction (PCR) was used for monitoring CMV DNA levels in HSCT patients weekly during the pre-engraftment period, with ganciclovir used as first line pre-emptive therapy if there was a possible active CMV infection, and then the patient was excluded from the study. During severe neutropenia (ANC <100 cells/ μL), all patients were isolated in single rooms with positive pressure, laminar air flow, and high-efficiency particulate air filtration. After the ANC exceeded 100 cells/ μL some of the clinically stable patients were moved to the intensive care department, which had 2 patients to a room and positive air pressure. The institution's standard protocols of initial empirical antibiotic therapy for febrile neutropenia included cephalosporins (cefepime or cefoperazone/sulbactam) or carbapenems (imipenem/cilastatin or meropenem) depending on the patient's risk group. Vancomycin was added in cases where there was a possible infection caused by gram-positive pathogens [9].

Before the administration of antibacterial therapy, in every patient with febrile neutropenia 10ml of blood was taken both from the peripheral vein and the central venous catheter with standard aseptic precautions and

cultivated in aerobic/anaerobic bioMerieux BacT/ALERT culture media in a BacT/ALERT 3D automated microbial detection system until a positive result was received or it reached the 7th day. In the case of a positive result, the microbial culture was isolated and grown on different manufactured culture media. Identification and antimicrobial susceptibility testing was performed using a bioMerieux VITEK 2 automatic system, while ESBL phenotype was detected via a VITEK 2 ESBL Test System. Additional antimicrobial susceptibility for carbapenem-resistant strains (resistance to: imipenem, meropenem, and doripenem) was confirmed by means of E-tests and disc-diffusion assay. The MIC breakpoints used for susceptibility testing were taken from the latest Clinical and Laboratory Standards Institute (CLSI) data [10]. The following clinical criteria of the isolated microorganism were taken: clinical signs of active infection and the isolation of the same microorganism with identical antimicrobial resistance profile from more than one blood culture media or more than once during a one-week period.

Methods of non-parametric statistics for both categorical (Chi-squared or Fisher's exact tests) and quantitative (Mann-Whitney U-test, Odds Ratio) methods were used in the statistical analysis. The distribution of the variable was determined by the Shapiro-Wilk test. Multivariate analysis was performed using logistic regression methods for categorical variables with $p \leq 0.2$ in previously performed univariate analysis. Data processing and analysis was performed using MedCalc Statistical Software v.14.10.2 (MedCalc Software bvba, Ostend, Belgium) and results were regarded as statistically significant when $p < 0.05$.

During the study period, a total of 360 transplantations were performed at our center, of which 62 were allogeneic and 298 were autologous. Positive results of blood cultures were obtained in 135 patients, leading to an overall incidence of BSI of 37.5%. 30-days mortality in patients with BSI in the pre-engraftment period after HSCT was 31.1 %. The median day of the first febrile episode with subsequently confirmed and BSI (N=135) was day 5 (interquartile range 4-7 days) from the HSCT. Table 1.1 shows the baseline demographic and clinical characteristics of the patients included in the study.

Table 1.2 describes the most significant characteristics of patients in connection with 30-days mortality in univariate analysis. Risk factors, which have shown statistical significance in univariate analysis, were subsequently checked for independence in multivariate analysis performed using logistic regression.

Table 1.1: The demographical and clinical baseline characteristics of patients with BSI in the pre-engraftment period after HSCT (N=135).

Baseline characteristics	N (%)
Age (years, median, interquartile range)	44 (32-53)
Gender (male)	64 (47.4)
Type of HSCT:	
Autologous	84 (62.2)
Allogeneic	51 (37.8)
Conditioning regimen:	
Myeloablative	44 (32.6)
Non-myeloablative/reduced intensity	91 (67.4)
Primary diagnosis:	
Acute myeloid leukemia	65 (48.2)
Hodgkin's lymphoma	23 (17.0)
Multiple myeloma	23 (17.0)
Non-Hodgkin's lymphoma	14 (10.4)
Acute lymphoblastic leukemia	6 (4.5)
Chronic myeloid leukemia	1 (0.7)
Myelodysplastic syndromes	3 (2.2)
Level of neutropenia:	
<100 cells/ μ L	89 (65.9)
100-500 cells/ μ L	28 (20.7)
>500 cells/ μ L	18 (13.3)

Table 1.2: Risk factors for fatal outcomes in adult patients with BSI in the pre-engraftment period after HSCT in univariate analysis.

Risk factor	30-days outcome		Total number (N=135)	Odds ratio (95% confidence interval)	<i>P</i>
	Alive (N=93)	Adverse outcome (N=42)			
Age ≥55 years	19 (20.4%)	11 (26.2%)	30 (22.2%)	1.38 (0.59–3.24)	0.4571
Male	41 (44.1%)	23 (54.8%)	64 (47.4%)	1.53 (0.74–3.19)	0.2514
Isolation of:					
Carbapenem-resistant <i>A. baumannii</i> or <i>P. aeruginosa</i>	6 (6.5%)	17 (40.5%)	23 (17.0%)	7.37 (2.54–21.35)	0.0002
ESBL-producing <i>Enterobacteriaceae spp.</i> ^{a)}	18 (19.4%)	6 (14.3%)	24 (17.8%)	0.43 (0.15–1.21)	0.1105
MRSA ^{b)}	11 (11.8%)	4 (9.5%)	15 (11.1%)	0.52 (0.15–1.77)	0.2936
Inadequacy of empirical antibacterial therapy	9 (9.7%)	29 (69.1%)	38 (28.2%)	20.82 (8.06–53.78)	<0.0001
Acute myeloid leukemia	37 (39.8%)	28 (66.7%)	65 (48.2%)	3.03 (1.41–6.5)	0.0045

^{a)} *Escherichia coli* or *Klebsiella pneumoniae*, showing the resistance phenotype of extended spectrum beta-lactamase producer;

^{b)} Methicillin-resistant *Staphylococcus aureus*.

All of the patients included in the study had a complete remission of their primary disease at the start of HSCT, so the disease stage was not included in the analysis. Therefore, the statistically significant risk factors for 30-days mortality in adult patients with BSI received HSCT in univariate analysis were the inadequacy of empirical antibacterial therapy (OR 20.82; 95% CI 8.06–53.78; $P < 0.0001$) and the isolation of carbapenem-resistant *Acinetobacter baumannii* or *Pseudomonas aeruginosa* (OR 7.37; 95% CI 2.54–21.35; $P = 0.0002$). The group of patients with acute myeloid leukemia were also at higher risk of an adverse outcome (OR 3.03; 95%

CI 1.41–6.5; $P=0.0045$). However, this group was analyzed thoroughly to eliminate possible confusion, and there were 4 patients with both a fatal outcome and an absence of autopsy data, who have had minimal residual disease (MRD) confirmed, so this data was not included in subsequent multivariate analysis. Older patients (≥ 55 years), as well as male patients, did not have a statistically significant increase in their risk of adverse outcomes in univariate analysis. The isolation of MRSA or ESBL-producers was also not a risk factor for 30-days mortality in multivariate analysis. The results of the subsequently performed multivariate analysis show that infection caused by carbapenem-resistant *Pseudomonas aeruginosa* or *Acinetobacter baumannii* is an independent risk factor for 30-days mortality in patients with BSI in pre-engraftment period after HSCT (regression coefficient 1.697; standard error 0.68; $P=0.0126$). The inadequacy of empirical antibacterial therapy was also statistically significant with an independent risk factor of 30-days mortality in the previously mentioned group of patients (regression coefficient 2.71; standard error 0.57; $P<0.0001$). Results of the multivariate analysis are shown in Table 1.3.

Table 1.3: Results of the multivariate analysis of risk factors for 30-days mortality in patients with BSI in the pre-engraftment period after HSCT.

Risk factor	Odds ratio (95% confidence interval)	<i>P</i>
Inadequacy of empirical antibacterial therapy	15.04 (95% CI 4.88-46.37)	<0.0001
Isolation of carbapenem-resistant <i>A. baumannii</i> or <i>P. aeruginosa</i>	5.46 (95% CI 1.44-20.7)	0.0126

Microbiological data concerning the causes of BSI in the pre-engraftment period after HSCT has shown the major impact of Gram-negative bacterial flora. Table 1.4 shows the spectrum of bacteria that caused BSI in the study.

Table 1.4: Causes of bloodstream infections in the pre-engraftment period after HSCT

Pathogen	N	Frequency of isolation (%)
<i>Klebsiella pneumoniae</i>	34	25.2
<i>Escherichia coli</i>	25	18.5
<i>Acinetobacter baumannii</i>	16	11.8
<i>Pseudomonas aeruginosa</i>	12	8.9
<i>Stenotrophomonas maltophilia</i>	1	0.7
<i>Staphylococcus epidermidis</i>	11	8.2
<i>Staphylococcus aureus</i>	23	17.0
<i>Staphylococcus hominis</i>	4	3.0
<i>Staphylococcus haemolyticus</i>	3	2.2
<i>Streptococcus pneumoniae</i>	2	1.5
<i>Enterococcus faecium</i>	2	1.5
<i>Enterococcus faecalis</i>	2	1.5

Therefore, among the causes of BSI in adult patients after HSCT, 65.2% was due to Gram-negative microorganisms and when these occur with non-fermenters (*A. baumannii*, *P. aeruginosa*, and *S. maltophilia*), the figure is 21.5% in the total etiological spectrum.

Among the *Klebsiella pneumonia* isolates (N=34), 18 (52.9%) have showed resistance to cephalosporins, while 7 (20.6%) were resistant to carbapenems. Among *Escherichia coli* isolates (N=25), only 6 (24.0%) were resistant to cephalosporins, and there was no resistance to carbapenems detected in *Escherichia coli* isolates. The level of carbapenem-resistance among Gram-negative non-fermenting bacteria (N=29) was shown to be extremely dangerous: 12 out of 16 *Acinetobacter baumannii* isolates, 10 out of 12 *Pseudomonas aeruginosa* isolates, and 1 isolate of *Stenotrophomonas maltophilia* have demonstrated resistance to carbapenems. Among the isolated strains of *Staphylococcus aureus* (N=23), 15 (65.2%) have demonstrated a phenotype of methicillin-resistant *Staphylococcus aureus*, which should be taken into account if anti-gram-positive empirical antibacterial coverage is indicated. Among 18 isolated coagulase-negative *Staphylococci*, only 5 (27.8%) showed resistance to oxacillin. The low level of isolation of *Streptococcus*

pneumoniae and *Enterococcus spp.* in the conducted study makes it difficult to discuss levels of antibiotic resistance in post-HSCT patients.

Inadequate empirical antimicrobial therapy in intensive care units is known to be associated with excess mortality [11]. However, the clinical impact in patients with BSI in the pre-engraftment period after receiving HSCT is still debated due to the limited number of observations. The completed study shows the clinical significance of adequate empiric antibacterial therapy in adult patients who received HSCT, and the high level of 30-days mortality in patients with an inappropriately chosen antibiotic when suffering from febrile neutropenia. This shows the importance of a knowledge of the local spectrum of pathogens in the center, as it may be helpful when choosing the right empiric antibacterial therapy regimen. The high rate of Gram-negative pathogens in our study corresponds with a similar trend in some European countries [9, 12]. This was the cause of the high rate of fatal outcomes in patients with BSI (31.1%) in the study which, when compared to similar studies showing mortality in range of 15.0–20.0% [5, 13], may be due to the local outbreak of metallo-beta-lactamase that produced *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. This should be investigated more thoroughly with the help of molecular methods, such as multilocus sequence typing. A significant risk factor for 30-days mortality was the isolation of the carbapenem-resistant *A. baumannii* or *P. aeruginosa* and so this should be an alert for a clinician to start the intense antibacterial therapy, including adding colistin to an adequate dosing regimen.

The main limitation of this study was the relatively small sample (N=135) but, with regard to the cost of every HSCT procedure, such a small number of observations may be important. The other limitation was that we used a definition of the inadequacy of empirical antibacterial therapy which was based on susceptibility, and antibiotics could still be clinically effective in some cases using in vivo. Also, it was not always possible to confirm if the main cause of a fatal outcome was a bloodstream infection. Finally, this study was conducted in one clinical center, but it is important to mention that this center performs HSCT for patients from all parts of the country. In conclusion, the risk factors for fatal outcome in adult patients with BSI in the pre-engraftment period after HSCT are the inadequacy of empirical antimicrobial therapy and the isolation of carbapenem-resistant *A. baumannii* or *P. aeruginosa*.

Additionally, febrile neutropenia (FN) remains one of the most common complications in HSCT patients. Bloodstream bacterial infections remain a

common cause of FN in neutropenic patients. The choice of an initial strategy for antibacterial treatment in FN patients is based mainly on clinical and epidemiological risk factors because of the low frequency of culture isolation and reduced clinical manifestations of infection. The aim of the next study was to determine the risk factors for febrile neutropenia or microbiologically proven bloodstream infection in adult patients receiving HSCT. 242. Patients undergoing allogeneic or autologous HSCT at the Belarus National Centre for Hematology and Bone Marrow Transplantation from January 2013 to January 2015 were monitored and their clinical data was reviewed. The age range of the patients included in this study was 18–65 years: 42% of them were male, 58% female. The primary outcome was an episode of FN (fulfilled criteria created by Freifeld et al., 2011), while the secondary outcome was microbiologically proven bacterial bloodstream infection (BSI). The isolation of pathogens was performed by standard means using BacT/ALERT Standard Aerobic/Anaerobic bottles and the BacT/ALERT 3D automated microbial detection system. Identification and antibiotic resistance was studied with a VITEK 2 system and disc-diffusion methods. Categorical variables were analyzed with χ^2 test and Fisher's exact test, and continuous variables were analyzed with the Mann-Whitney U test and Odds Ratio. A multivariate analysis with logistic regression was conducted for the categorical variables with P-value $\leq 0,2$ in a previously performed univariate analysis. A significant P-value was considered to be $< 0,05$. There were 87 patients with episodes of FN, and the incidence of FN in HCST recipients was 36%. Among them 39 patients had microbiologically proven BSI: i.e. 16% of all HSCT recipients or 45% of those who had FN. Most of the cases of BSI were caused by *E. coli*, *Kl. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *Streptococcus spp.* Some of the independent statistically significant risk factors for both FN and BSI are as follows: profound neutropenia (OR 2,34, 95% CI 1,19-13,24, $p=0,012$ for FN; OR 2,44, 95% CI 1,96-9,54, $p=0,005$ for BSI); neutropenia duration >14 days (OR 1,37, 95% CI 1,08-12,93, $p=0,049$ for FN; OR 1,68, 95% CI 1,14-8,73, $p=0,045$ for BSI); and active main disease at the start of the HSCT procedure (OR 3,41; CI 2,32-8,63, $p=0,01$ for FN; OR 1,28, CI 1,04-3,81, $p=0,049$ for BSI). Prior to HSCT patient colonization with ESBL-positive *Enterobacteriaceae spp.* and previous ICU hospitalization had statistical significance as potential risk factors of BSI, which may be proved by using a larger number of patients in future studies (OR 1,64, 95% CI 0,89-4,36, $p=0,64$ for colonization; OR 2,31, 95% CI 1,27-6,41, $p=0,72$ for ICU hospitalization).

Therefore, the above risk factors and most common pathogens should be taken into account when choosing a clinical approach to empiric antibacterial treatment and prophylaxis in adult HSCT patients.

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CHAPTER TWO

INVASIVE PULMONARY ASPERGILLOSIS AS A TREATMENT CHALLENGE IN HEMATOLOGY

Invasive aspergillosis remains a common cause of infectious complications in immunocompromised patients. In patients receiving hematopoietic stem cell transplantation (HSCT) invasive aspergillosis is one of the most frequent causes of pneumonia-related mortality. The incidence of invasive aspergillosis in adult patients after HSCT varies from 5.7% to 10.5% [1–3]. Profound neutropenia, corticosteroid therapy, and graft versus host disease have been listed as risk factors associated with invasive aspergillosis in HSCT patients [4–5]. We will present a patient with a history of probable invasive aspergillosis, which is resistant to voriconazole, after tandem autologous HSCT to treat Hodgkin's lymphoma. Next, in order to better understand this clinical issue, we will present a clinical case and the results of a chest CT X-ray, sputum microscopy, and photos of cutaneous aspergillosis.

A 37-year-old man with Hodgkin's lymphoma presented to the hospital with fever (38,0–38,5°C), fatigue, increasing shortness of breath, and a dry cough with a small amount of clear sputum. This condition was maintained for 3 days until his admission to hospital. During the clinical investigation, it was found that he had weakened breathing on auscultation (mainly on the left) with no rhonchi or crepitation present. His blood pressure was 110/70 mmHg, and his heart rate was 82 beats per minute. His heart sounds were regular without extra sounds. The patient had a 2-year medical history of Hodgkin's lymphoma, and he had undergone a tandem autologous HSCT (2 HSCT with a 4-months interval) with a BEAM conditioning regimen. He had also received 8 courses of high-dose chemotherapy (ABVD regimen), 2 dexaBEAM courses, and 1 DHAP course with collection of hematopoietic stem cells. At the time of his hospital admission, he was receiving oral fluconazole prophylaxis.

Significant laboratory parameters included the leukocytosis of $11,800 \text{ cells/mm}^3$ with a slight neutrophilic left shift; the erythrocytes were $3,8 \text{ mln/mm}^3$; and hemoglobin was 100 g/l . The level of platelets, urea, total protein, glucose, ALT, AST, K^+ , Na^+ , Cl^- , amylase, GGTP, alkaline phosphatase, and LDH were all in the normal range. Urine analyses were normal. The patient had elevated C-reactive protein on admission at 95 mg/l . Multiple blood cultures remained negative. Serum procalcitonin was also negative. Galactomannan in the blood showed a positive result ($\text{I}=8,93$). Multiple sputum culture showed no growth. Sputum microscopy with a gram staining was performed and the microscopic features (hyphae) of *Aspergillus spp.* were found (Figure 2.1).

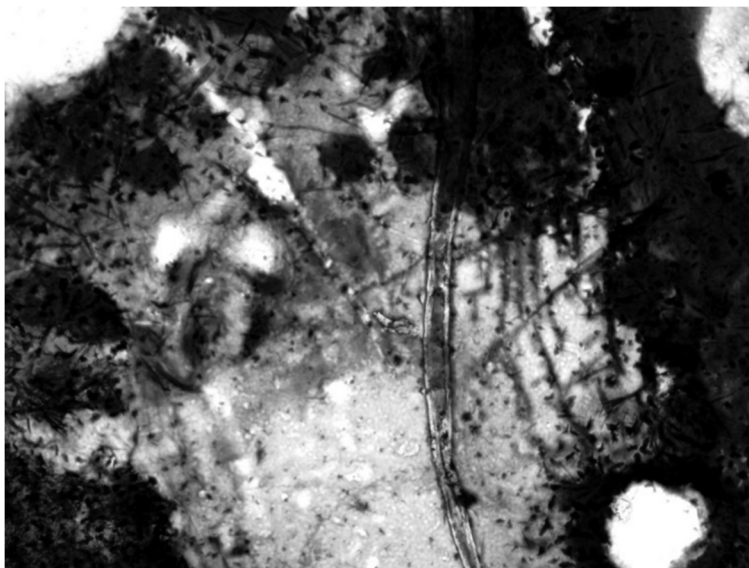


Figure 2.1. Sputum microscopy photography from a patient with a probable invasive aspergillosis

A chest X-ray was performed and showed airspace opacity on the left side. A follow-up CT scan of the chest showed ground-glass opacity (halo sign) on the left with an air crescent sign (Figure 2.2). The halo sign (HS) in chest imaging is a feature seen on lung window settings, as a ground glass opacity surrounding a pulmonary nodule or mass indicates a hemorrhage. It is typically seen in invasive aspergillosis. Histopathologically, it represents a focus of pulmonary infarction surrounded by alveolar hemorrhage. An air crescent sign describes the crescent of air that can be

seen in invasive aspergillosis and is usually the result of increased granulocyte activity [6–8].

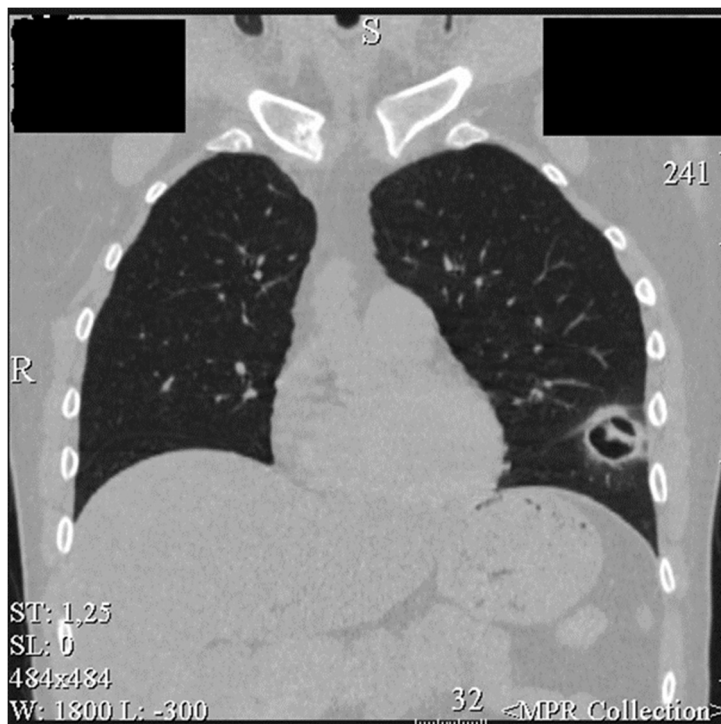


Figure 2.2. Thoracic CT-scan image of a patient with a probable invasive aspergillosis

Based on sputum microscopy, a CT-scan, clinical examination, and a positive serum galactomannan, we decided to treat the patient for probable invasive aspergillosis. The definition of probable aspergillosis requires the fulfillment of criteria within 3 categories: host factors, clinical manifestations (symptoms, signs, and radiological features), and microbiological evidence. With 2 important exceptions, proven or probable infection requires the recovery of an organism. The first exception includes the fairly frequent occurrence of histopathological demonstration of hyphae, which is consistent with the aspergillus species in patients with negative culture results. The other exception consists of fulfilling the diagnostic criteria for probable invasive aspergillosis with a surrogate non-culture based method (e.g., a positive galactomannan assay or b-glucan assay result and

radiologically compatible CT findings) in an immunocompromised host with clinical findings of infection that constitute the definition of probable invasive aspergillosis [9]. The patient's treatment began with voriconazole (6mg/kg IV every 12h for 1 day, followed by 4mg/kg IV every 12h). After 2 weeks of treatment the clinical condition of the patient remained stable, he was febrile up to 38°C, the dry cough and fatigue remained, and the levels of galactomannan did not decrease. The decision was made to change the treatment to caspofungin, which had a significant clinical effect on the 3rd day. Serum galactomannan became negative on the 10th day of the caspofungin treatment. No sputum or blood culture showed microbiological growth, the thoracic CT-scan showed slight improvement, and surgical treatment of any possible remaining lung defect was scheduled. The patient stayed on caspofungin for 12 weeks before changing to fluconazole prophylaxis.

As clinical signs and symptoms are not specific for the diagnosis of aspergillosis, radiographic imaging is critical. The role of imaging is to identify the site of infection, to assess the type, number and size of lesions, and its local extension. Imaging also helps to direct diagnostic procedures (e.g., BAL or CT-guided biopsy) to the most appropriate area. It is important to understand the main radiographic symptoms of an aspergilloma. The characteristic chest radiographic appearance of an aspergilloma is that of a round or oval mass with the opacity of that of a soft-tissue mass. Often, an adjacent crescent-shaped air space (i.e., the air-crescent sign) separates the fungal ball from the cavity wall. This is illustrated on Figures 2.3 and 2.4 (from real patients' CT scans; courtesy of the author).

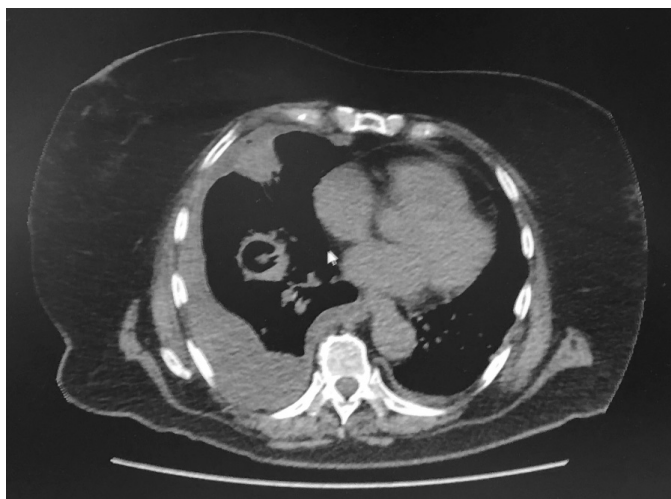


Figure 2.3. Thoracic CT-scan image of a patient with aspergilloma (soft tissue opacity)



Figure 2.4. Thoracic CT-scan image of a patient with aspergilloma (air-crescent sign)

Invasive fungal infections are frequent causes of morbidity and mortality in adult patients receiving HSCT. Without adequate therapy, invasive

pulmonary aspergillosis will almost always progress to a fatal pneumonia in patients who have received HSCT. This pneumonia may be characterized by pulmonary hemorrhagic infarction or progressive necrotizing pneumonia. Voriconazole is recommended for most patients with aspergillosis. For patients who are intolerant of or refractory to voriconazole, therapeutic options include a change of class using an AMB lipid formulation or an echinocandin [9]. A study into the use of caspofungin for patients who are intolerant of or refractory to conventional therapy demonstrated a favorable response rate of 45%, with higher responses (50%) in patients with invasive pulmonary aspergillosis comparing to patients with disseminated aspergillosis (23%) [10]. One of the important clinical forms of aspergillosis is a cutaneous form (Figure 2.5: real patients' CT scan, courtesy of the author), which may be combined in some cases with an invasive pulmonary aspergillosis.

Therefore, a high incidence of invasive aspergillosis in HSCT patients should be kept in mind when dealing with transplant patients. Even though culture isolation is not always possible, other clinical and laboratory tests (galactomannan, CT-scan, sputum microscopy) may be useful to diagnose aspergillosis. Voriconazole remains a treatment of choice for patients with invasive aspergillosis, along with the possibility of using echinocandins in refractory cases.



Figure 2.5. Cutaneous aspergillosis in a patient with leukemia, who is having chemotherapy.

Prophylaxis of aspergillosis is recommended with posaconazole, voriconazole, and/or micafungin during prolonged neutropenia for those who are at high risk of invasive aspergillosis. Prophylaxis with caspofungin is also likely to be effective. Prophylaxis with itraconazole is effective but therapy may be limited by absorption and tolerability. Triazoles should not be co-administered with other agents known to have potentially toxic levels with concurrent triazole co-administration (e.g., vinca alkaloids and others).

Empiric antifungal therapy is recommended for high-risk patients with prolonged neutropenia who remain persistently febrile despite broad-spectrum antibiotic therapy. Antifungal options include a lipid formulation of AmB, an echinocandin (caspofungin or micafungin), or a voriconazole. In the case of non-effective treatment, there is an individualized approach available as a salvage therapy that takes into consideration the rapidity, severity, and extent of infection, as well as patient comorbidities and the need to exclude the emergence of a new pathogen. The general strategies for salvage therapy typically include changing the class of antifungal, tapering or reversal of underlying immunosuppression when feasible, and surgical resection of necrotic lesions in selected cases. In the context of salvage therapy, an additional antifungal agent may be added to the current therapy or a combination of antifungal drugs from different classes, other than those in the initial regimen, may be used. In patients currently receiving antifungal treatment and exhibiting an adverse event attributable to this agent, it is recommended that they are given an alternative class of antifungal, or the use of an alternative agent with a non-overlapping side-effect profile [9, 11].

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CHAPTER THREE

SEPSIS BIOMARKERS IN HEMATOPOIETIC STEM CELLS TRANSPLANTATION

Bacterial bloodstream infections (BSI) remain one of the leading causes of infectious complications after HSCT, and occur in approximately 5–10% of autologous and 20–30% of allogeneic HSCT recipients [1]. Despite the improved level of supportive care the mortality rate due to BSI remains significant: from 24 to 40% in allogeneic HSCT [2–6]. Traditionally, the diagnosis of BSI includes the results of culturing techniques. Positive blood culture is known to be the most certain method of diagnosis but it has a number of limitations. For instance, in a large percentage of patients it remains negative despite the typical clinical presentation of sepsis [7]. The other issue of standard culturing techniques is that it still takes significant time for the laboratory to give the results to the doctor. It is well known that adequate and on-time prescribed antimicrobial therapy is a key to success in patients with BSI [8]. However, there is a number of cases when it is not clear whether the febrile episode in a concrete patient is a symptom of BSI or has any other cause (e.g. viral or fungal infection, reaction to chemotherapy infusion, or a reactivation of hematologic disease). In patients receiving HSCT, the consequences of BSI may be dramatic when taking into account the level of immunosuppression caused by high-dose chemotherapy and total body irradiation. The other issue, which may affect the early diagnosis of BSI in HSCT patients, is the possibility of having a potentially fatal BSI with mild clinical symptoms of infection in such patients. Although, the clinical significance of sepsis biomarkers increases in HSCT recipients.

Among widely used biomarkers which had been studied in neutropenic patients are procalcitonin (PCT), C-reactive protein (CRP), and interleukin-6 [9–11]. Despite this fact, the use of biomarkers in neutropenic patients remains a controversial question; for instance, the guidelines of the Infectious Diseases Society of America do not include the use of biomarkers in their recommendations [12]. However, existing studies are based on a small samples of patients receiving HSCT in a total

group of neutropenic patients, so there is not enough data to be sure about the diagnostic and clinical significance of those biomarkers in HSCT recipients [13, 14]. Previously, it has been shown that biomarkers are not equally effective in special groups of patients as important differences in the diagnostic characteristics of presepsin were present in advanced forms of acute kidney injury of if they were on hemodialysis, thereby indicating a need for different cut-off values in these particular groups [15, 16]. Furthermore, there is no compelling information concerning the usefulness of presepsin in adult patients after HSCT, and there is a practical need for results from a comparative analysis of diagnostic parameters for PCT, CRP, and presepsin in HSCT recipients [17, 18]. The continuing emergence of Gram-negative pathogens as a cause of BSI affects transplant centers worldwide, so the use of biomarkers in patients after HSCT should be reevaluated according to this recent shift from gram-positive microorganisms [3, 19, 20]. Therefore, it is important to assess and compare the diagnostic value of presepsin, procalcitonin, and C-reactive protein as early biomarkers of a Gram-negative bacterial bloodstream infections in HSCT recipients.

The main objective of the study was to identify the diagnostic value of presepsin, procalcitonin, and C-reactive protein, as well as to perform a comparative analysis of those biomarkers in a group of HSCT recipients with Gram-negative bacterial bloodstream infections. Data relating to age, gender, date, and type of transplantation; conditioning chemotherapy regimen; and microorganisms isolated from blood and antibacterial therapy were prospectively collected in hematopoietic stem cell recipients in this observational clinical study. There were 52 adult patients who had undergone autologous or allogeneic HSCT with neutropenia and all of them were inpatients. The study was performed between January 2013 and October 2015. An inclusion criterion in the study was that it only involved adult patients with febrile neutropenia during the 30 days (pre-engraftment period) after autologous or allogeneic HSCT. Febrile neutropenia was assessed using the definition created by Freifeld et al.: a single oral temperature measurement of $>38.3^{\circ}\text{C}$ or a temperature of $>38.0^{\circ}\text{C}$ sustained over a 1-hour period with an absolute neutrophil count in peripheral blood (ANC) of ≤ 500 cells/mm³ or an ANC that is expected to decrease to ≤ 500 cells/mm³ during the next 48 hours [12]. The exclusion criteria included diabetes mellitus, acute kidney injury (clinically and/or laboratory confirmed), and acute heart failure. Patients who had received anti-thymocytic immunoglobulin during 7 days before the onset of febrile episode were excluded from the study. Bloodstream infection was defined as having a microbiologically proven growth from a blood culture of a